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Combination of Nonwoven Filters and Mesenchymal Stem Cells Reduced Glomerulosclerotic Lesions in Rat Chronic Kidney Disease Models

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Abstract

Background and Objectives: The increasing incidence of patients requiring hemodialysis has become a medical and economic problem globally; it is necessary to maintain renal glomerulus functionality to prevent the progression of chronic kidney disease. As a therapeutic tool for preventing the progression of chronic kidney disease, mesenchymal stem cells (MSCs) are a promising source of both growth factors and cells for regeneration. However, the escape of MSCs from injection sites, as well as the insufficient production of growth factors, is issues that remain unresolved. In the present study, a complex of cells and a nonwoven filter was localized in an injured kidney to provide growth factors such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF). **Methods and Results**: Nonwoven biodegradable polylactic acid (PLA) filters were used to capture rat bone marrow stem cells (r-BMSCs). The capture rates of r-BMSCs on five PLA filter disks were over 85%. The production of HGF by r-BMSCs on PLA filters was markedly enhanced through interactions between the cells and the nonwoven filter. Conversely, the production of VEGF by r-BMSC on PLA filters was unchanged. Complexes of nonwoven filters and cells were implanted onto the surfaces of kidneys of 5/6-nephrectomized rats, which are characterized by progressive glomerulosclerosis. Within the r-BMSC/PLA complexes, deleterious changes in serum creatinine levels were not attenuated. However, PLA filters with r-BMSCs, which enhanced HGF production over 4 weeks of cul-

ture, significantly (P = 0.03) decreased urinary protein levels at 4 weeks after implantation compared to untreated nephrectomized rats. Further histopathological studies revealed that glomerulosclerotic lesions were significantly (P = 0.008) reduced by treatment with the r-BMSC/PLA complex. **Conclusion**: Devices made of PLA nonwoven filters and stem cells are potentially useful for the prevention and treatment of chronic kidney disease.

Keywords

Nonwoven Filter, Mesenchymal Stem Cell, Hepatocyte Growth Factor, Regenerative Medicine, Chronic Kidney Disease

1. Introduction

Over 26 million people worldwide have undergone renal replacement therapy, including hemodialysis; this increase in hemodialysis patients has become a medical and economic problem worldwide [1] [2]. During the progression of kidney diseases, focal and segmental sclerotic changes in the glomeruli have been observed due to glomerular hypertension, promoting renal damage. This finding has been shown to be independent of underlying diseases and is known to be a common pathway. Thus, in order to prevent the progression to end-stage kidney disease, it is important to suppress the pathway leading to glomerulosclerosis [3].

Vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) have been reported to be important for kidney regeneration [4]. Previous studies have revealed that injections of VEGF can prevent glomerulosclerotic injury in rats [5], and that injections of HGF can ameliorate chronic kidney disease (CKD) in rodents [3] [6]. However, the delivery of the appropriate concentrations and combinations of growth factors to the kidney at the appropriate times is difficult when using exogenous treatments. Furthermore, the short half-life of HGF precludes the ability to maintain high blood concentrations of this factor [7]. Several studies have shown that the intravenous administration of mesenchymal stem cells (MSCs) can ameliorate CKD or acute kidney injury [8] [9] [10] [11]. However, intravenously injected MSCs were ineffective, as MSCs did not successfully reach and remain in the injured site [12]. Therefore, the injection of extrinsic MSCs and growth factors may not be the most adequate method to deliver MSCs and growth factors to an injured kidney.

In this study, we focused on nonwoven filters as a tool for the localization of MSCs near the lesion area. Nonwoven filters are widely used to remove leukocytes from blood for two purposes. They are either used for the removal of leukocytes from donated blood in order to prevent post-transfusion graft versus host disease, or to treat ulcerative colitis by removing leucocytes from systemic circulated blood. Therefore, nonwoven filters may be suitable for the capture of MSCs and circulating mononuclear cells (MNCs), as well as their subsequent

applications in regenerative medicine. We previously created novel devices with nonwoven filters for regenerative medicine using these filters as a tool to localize the cells to the injured site. In our previous studies, nonwoven filter disks composed of polylactic acid (PLA) fibers with fiber diameters of 1.8 μm effectively captured MSCs and peripheral blood cells and enhanced the production of several growth factors. Specifically, human peripheral blood cells captured on nonwoven biodegradable PLA filters enhanced the production of VEGF, platelet-derived growth factor-AB, and transforming growth factor (TGF)-β1; furthermore, human MSCs captured on nonwoven biodegradable PLA filters enhanced the production of VEGF [13]. Further, as a therapeutic device, nonwoven filters that captured cells were used to enhance wound healing [14]. Briefly, mouse peripheral blood cells (m-PBCs), including MNCs, were captured on nonwoven filters in an appropriate housing. Then, the filters were removed from the housing and embedded into wounded skin areas so that the cells were located very close to the target site. These complexes of PLA nonwoven filters and m-PBCs promoted the healing of skin wounds in db/db mice, possibly as a result of the enhanced production of fibroblast growth factor-7 and/or TGF-β1 [14]. The cells captured on the filters provide a localized scaffold and may interact with the wound site to produce a suitable composition of growth factors and cytokines at the appropriate times.

In the present study, we adapted this technology to use nonwoven filters and cells for the treatment of CKD, as shown in **Figure 1**. Cells were captured on filters and subsequently placed near injured kidneys to provide a local supply of growth factors. Our basic strategy for renal regeneration was as follows: 1) appropriate cells could be localized around the kidney using nonwoven filters, 2) nonwoven filters could stimulate and enhance the production of growth factors

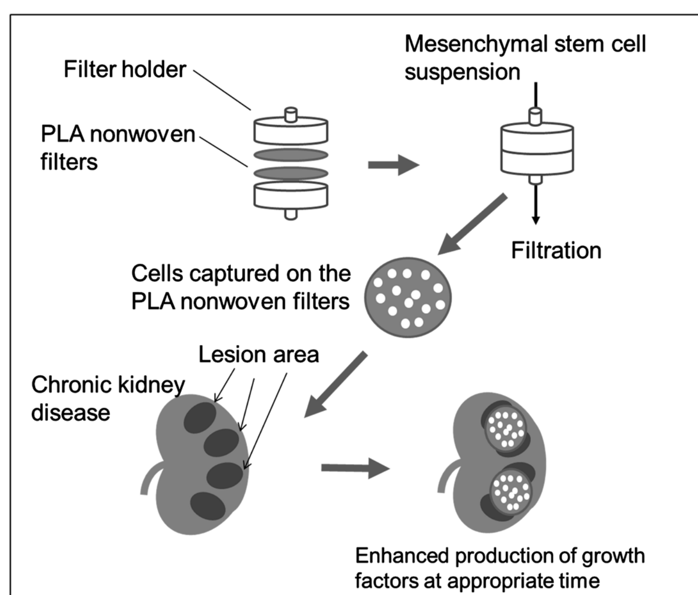


Figure 1. Schema of the strategy for renal regeneration using cell-capture devices with nonwoven filters.

and cytokines, and 3) cells and growth factors/cytokines could be provided from *in situ* cell-laden nonwoven filters at the appropriate times and concentrations.

2. Materials and Methods

2.1. Animal Ethics

This study was performed in accordance with the Regulations for the Management of Laboratory Animals at Fujita Health University, and all protocols were approved by the Institutional Animal Care and Use Committee at the Education and Research Facility of Animal Models for Human Diseases Center of Fujita Health University (Toyoake, Japan).

2.2. Nonwoven Filter Devices for Capturing Cells

Nonwoven filters made of PLA (fiber diameter, 1.8 μm; fiber density, 33 g/m^2) were provided by Asahi Kasei Fibers Corporation (Tokyo, Japan). Rat bone marrow mesenchymal stem cells (r-BMSCs) were purchased from Lonza Japan (Tokyo, Japan) and cultured according to the manufacturer's recommendations. Nonwoven filter disks of 13-mm in diameter were used for in vitro studies of growth factor production as previously reported [13]. For in vivo therapeutic studies of CKD rats, 25-mm filters were used for covering wider areas of the kidney surface. Devices with nonwoven filters for capturing cells were prepared as previously described [13]. Briefly, bundles of 13- or 25-mm nonwoven-filter disks were placed in Swinnex 13 or Swinnex 25 Filter Holders (Millipore, Billerica, MA, USA), respectively, and used to filter r-BMSCs suspensions in injection syringes.

The cell capture rate was defined as follows:

$$\text{Capture rate}(\%) = 100 \times \left(1 - \frac{C_a}{C_b}\right)$$

C_a, Cell count after filtration. C_b, Cell count before filtration.

2.3. Electron Microscopy

Electron microscopy was performed as described previously [13]. Briefly, filter disks with captured cells were fixed with 2.5% glutaraldehyde/0.05 M sodium phosphate (pH 7.4) for at least 24 h at 4˚C. Subsequently, filter disks were dehydrated in a graded ethanol series, followed by 100% t-butyl alcohol, and then freeze-dried at −5˚C using a freeze dryer (JFD-310, JEOL, Tokyo, Japan) and coated with gold using an ion sputtering device (JFC-1500, JEOL). Samples were then examined using a scanning electron microscope (S-2600N, Hitachi, Tokyo, Japan).

2.4. Measurements of Growth Factor Production by Captured Cells on Filters

To collect r-BMSCs, 13-mm diameter disks were placed in a Swinnex 13 Filter

Holder and washed with phosphate buffered saline (PBS); subsequently, 1-ml suspensions of 8×10^4 cells in culture media were filtered using the cell-capture devices. Nonwoven filters with captured r-BMSCs (r-BMSC/PLA group) were placed in 6-well plates, and 200-μl aliquots of 0.3 mg/ml type I collagen (Cellmatrix Type I-A, Nitta Gelatin, Osaka, Japan) were added.

Subsequently, complexes of r-BMSCs and nonwoven filters were cultured for 4 weeks in 3-ml aliquots of MSCGM Bullet Kit medium (Lonza) in a humidified atmosphere containing 5% CO_2 at 37˚C. The medium was changed every 3 - 4 days, and conditioned media were collected and combined each week. Growth factor concentrations were measured in conditioned media at 1 (0 - 7 days), 2 (7 - 14 days), 3 (14 - 21 days), and 4 (21 - 28 days) weeks after the start of culture. Control media were collected from cultures of r-BMSCs without nonwoven filters (control group) on plates coated with 200-μl aliquots of 0.3-mg/ml type I collagen. The concentrations of HGF and VEGF in conditioned media were measured using the Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA).

2.5. Rat CKD Model

Twelve-week-old male 5/6-nephrectomized rats (Weight 396 ± 30.5 g) with the right kidney removed, as well as the lower and upper one-third of the left kidney removed, were used as rat CKD models. Ten-week-old male 5/6-nephrectomized SD rats were purchased from Charles River Laboratories (Yokohama, Japan).

2.6. Implantation of Nonwoven Filters with Captured Cells into Rats with CKD

To collect r-BMSCs, 25-mm diameter disks were placed in a Swinnex 25 Filter Holder and washed with saline. Subsequently, 5-ml suspensions of 3×10^5 r-BMSCs in culture medium were filtered through the devices. Next, a ventrotomy was performed in 12-week-old 5/6-nephrectomized rats under isoflurane anesthesia. Nonwoven filters with captured cells (r-BMSC/PLA group), nonwoven filters without cells (PLA group), and cells without nonwoven filters (r-BMSC group) were then placed onto kidneys after gently removing the renicapsule. Filters were then fixed to adipose tissues around the kidneys using surgical sutures, and 500-μl aliquots of 0.3 mg/ml type I collagen were dropped onto the nonwoven filters. The control (untreated) group was comprised of untreated 5/6-nephrectomized rats.

2.7. Implantation of Nonwoven Filters with Captured Cells into Rats with CKD

To evaluate the extent of kidney injury, blood and urine samples were collected every 4 weeks after implantation surgery. Blood samples were collected from the caudal vein under anesthesia, and serum creatinine concentrations were determined using LabAssay Creatinine kits (Wako, Osaka, Japan). To determine urinary protein content, rats were placed gently in metabolic cages and urine sam-

ples were collected over 24 h in sample cups. Subsequently, urine samples were mixed thoroughly and assayed using the Quick Start Bradford protein assay (Bio-Rad Laboratories, Hercules, CA, USA).

2.8. Histopathological Evaluations of Kidneys

Eight weeks after implantation surgery, kidneys were resected from rats and fixed in 10% formalin. Next, paraffin-embedded sections stained with Periodic Acid-Schiff reaction (PAS) and Hematoxylin & Eosin (H&E) staining were used for microscopic analyses. The severity of glomerulosclerotic changes was determined by a renal pathology expert in a blinded manner. The evaluation was performed in all glomeruli in a single cross-section of the coronal plane of kidney in each rat. The incidence of glomerulosclerotic lesions was defined as follows:

$$\text{Incidence of glomerulosclerotic lesions}(\%) = 100 \times \frac{N_a}{N_b}$$

N_a, Number of glomeruli with segmental sclerotic changes.

N_b, Total number of observed glomeruli in a single section.

2.9. Statistical Analysis

All data are expressed as the mean ± standard deviation (SD). The differences in nonparametric continuous variables were identified using Wilcoxon's rank sum test using JMP10 software (SAS Institute, Inc., Cary, NC, USA). A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Growth Factors Produced by r-BMSCs on Nonwoven-Filter Disks

Five PLA nonwoven-filter disks captured 85.8% ± 11.9% of r-BMSCs (n = 3). Electron micrographs of nonwoven PLA filters without cells (**Figure 2(a)**) and filters with r-BMSCs (**Figure 2(b)**) were then analyzed. **Figure 2(b)** shows that r-BMSCs (white arrows) were captured mainly through adherence to fiber surfaces, rather than by filtration.

HGF and VEGF production by r-BMSCs on nonwoven PLA filters was measured over 4 weeks of culture. HGF production from r-BMSC/PLA complexes gradually increased during this period, and was significantly higher than in controls at 2, 3, and 4 weeks. At 4 weeks, HGF production was almost unchanged in controls and was 7.7-fold higher in the r-BMSC/PLA group (**Figure 2(c)**). In contrast, VEGF production by r-BMSCs on PLA filters was significantly suppressed compared to the control until 3 weeks (**Figure 2(d)**). However, VEGF production by r-BMSC/PLA complexes gradually increased and reached control levels at 4 weeks. VEGF and HGF concentrations in culture medium were <30.0 and <11.0 pg/ml, respectively.

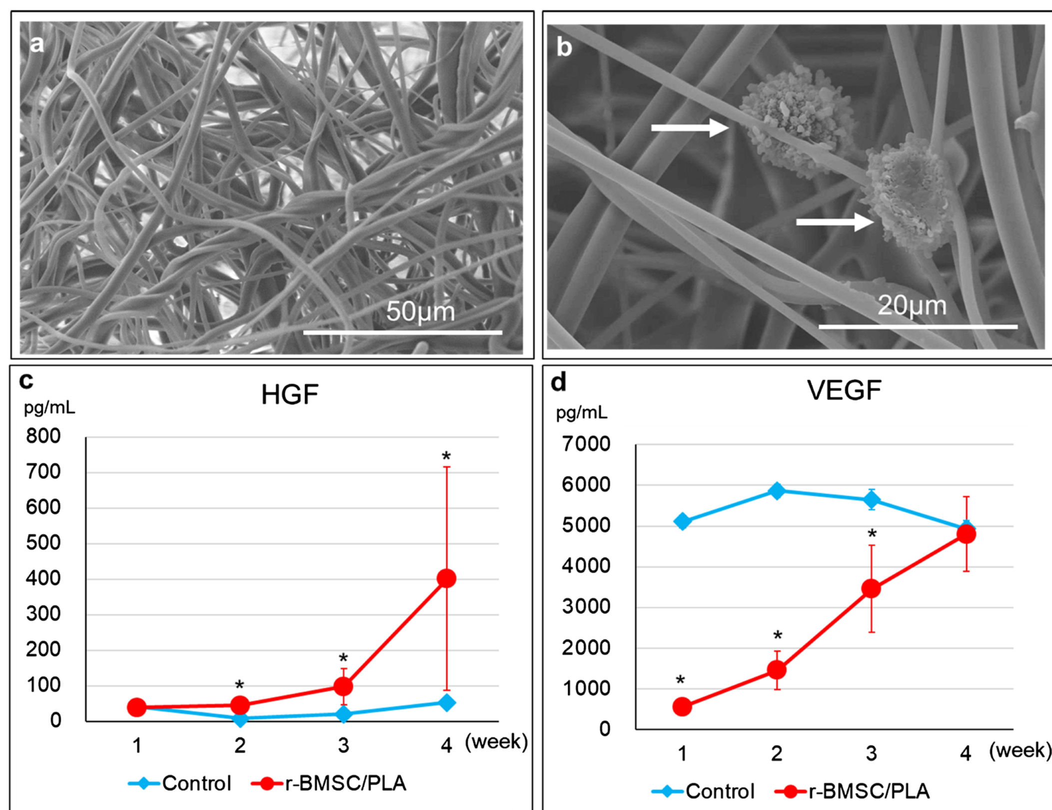


Figure 2. Complex of r-BMSCs on PLA nonwoven filters. (a) Scanning electron photomicrographs of PLA nonwoven filter disks only. (b) Scanning electron photomicrographs of r-BMSCs on PLA filter disks; white arrows indicate r-BMSCs adherent to the fibers. ((c), (d)) Production of growth factors by r-BMSCs on PLA filter disks at 4 weeks of culture. (c) HGF production; (d) VEGF production. Control, conditioned medium from r-BMSCs alone; r-BMSC/PLA, culture medium from r-BMSCs on five PLA filter disks; $^*p < 0.05$ vs. control group; n = 6 per group.

3.2. Effects of r-BMSCs with PLA Nonwoven Filters in CKD Rats

In this study, 5/6-nephrectomized rats were used as models of CKD. Five PLA nonwoven-filter disks (diameter: 25 mm) were used for capturing 3×10^5 r-BMSCs each. The cell/filter complexes, or filters without cells, were then implanted onto the kidney surfaces of 5/6-nephrectomized rats in the r-BMSC/PLA (n = 6) and PLA (n = 4) groups, respectively.

In the group implanted with r-BMSCs alone (r-BMSC, n = 4), suspensions of 3×10^5 r-BMSCs were implanted with type I collagen under the kidney capsule. Untreated 5/6-nephrectomized rats were used as controls (Untreated, n = 10). The urinary protein levels in the r-BMSC/PLA group (32.4 ± 19.6 mg/day) were significantly lower than those in the untreated group (68.1 ± 39.4 mg/day, $p = 0.03$) at 4 weeks after implantation, indicating that kidney function was improved in CKD rats (**Figure 3(a)**). Moreover, urinary protein levels in the r-BMSC/PLA group remained lower than those in the untreated group for 8 weeks after implantation. However, this difference was not significant ($p = 0.25$) (**Figure 3(a)**). In contrast to urinary protein levels, serum creatinine levels did not differ significantly between any of the experimental groups (**Figure 3(b)**).

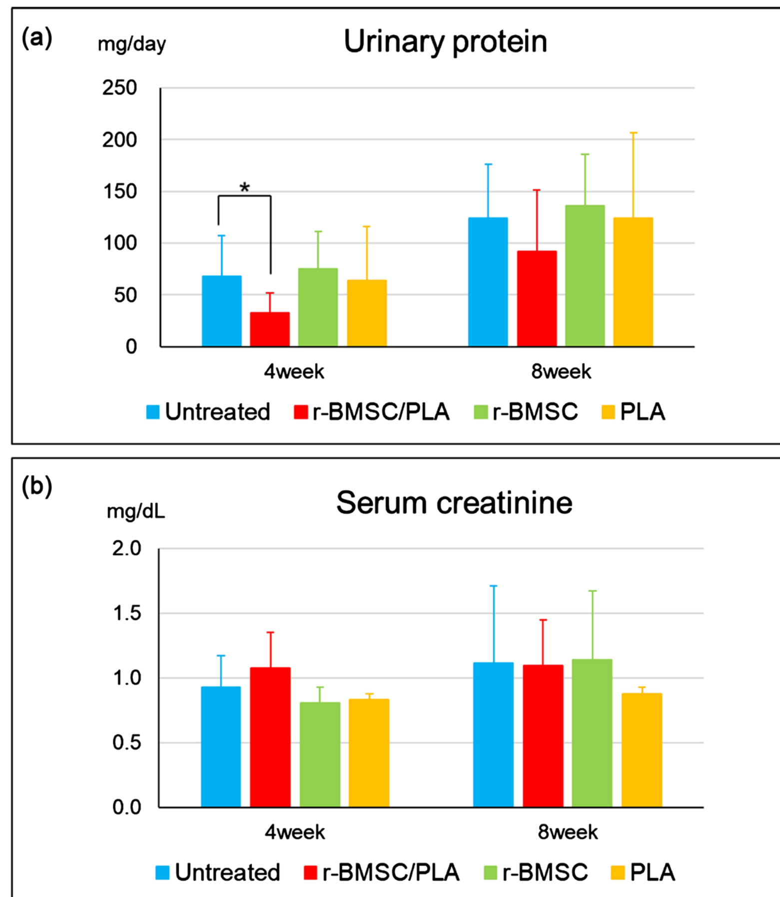


Figure 3. Therapy for 5/6-nephrectomized rats using cells on PLA nonwoven filters. PLA filter disks with or without r-BMSCs were implanted onto the kidneys of 5/6-nephrectomized rats. Control 5/6-nephrectomized rats received renal subcapsular transplants of r-BMSCs or remained untreated. (a) Analyses of urinary protein levels. (b) Analyses of serum creatinine levels. Untreated (n = 10), untreated 5/6-nephrectomized rats; r-BMSC/PLA (n = 6), 5/6-nephrectomized rats treated with r-BMSCs on five PLA filter disks; r-BMSC (n = 4), 5/6-nephrectomized rats treated with r-BMSCs; PLA (n = 4), 5/6-nephrectomized rats treated with five PLA filter disks; *$p < 0.05$ vs untreated group.

3.3. Histopathological Evaluation of Kidneys after Treatment with Nonwoven Filters and Captured Cells

Histopathological analyses of renal cortices at 8 weeks after implantation of PLA nonwoven filters with cells were performed to investigate the morphological improvements related to reduced urinary protein levels in the r-BMSC/PLA group (Figure 4). The right column of Figure 5 (blue rectangle in Figure 4) is an enlarged image of the glomerulus shown in the circle of the figures in the left column. As shown in Figures 5(a)-(d), focal and segmental glomerular sclerosis lesions (black arrows) were observed in all groups. The incidence of glomerulosclerotic lesions was significantly lower in the r-BMSC/PLA group (3.3% ± 3.0%) than in the untreated group (18.4% ± 17.7%; $p = 0.008$) (Figure 5(e)). Moreover, no significant reductions in lesion numbers were observed in the PLA (16.8% ± 13.6%, $p = 0.83$) or r-BMSC (23.3% ± 26.6%, $p = 0.94$) groups compared to the

untreated group (Figure 5(e)).

Next, the contact area of the PLA nonwoven filter and kidney was histopathologically investigated. Figure 6 shows microscopic images of the contact area of the PLA nonwoven filter and the kidney (red rectangle in Figure 4). Black arrows indicate polynuclear foreign body giant cells; these were observed in both the r-BMSC/PLA (Figure 6(a)) and PLA groups (Figure 6(b)), and there were no differences between the groups. The black arrowheads in Figure 6(a) and Figure 6(b) show lymphocytes at the contact area of the PLA nonwoven filters and kidney, as well as those infiltrating the renal cortex. In addition, the fragmented PLA nonwoven filter is indicated by a yellow arrow (Figure 6(a)). The degree of fragmentation of the PLA nonwoven filters was comparable between the two groups.

4. Discussion

The combination of r-BMSCs and PLA nonwoven filters improved or prevented glomerulosclerosis in 5/6-nephrectomy rats with CKD, which is characterized by progressive glomerulosclerosis [15]. Kidney regeneration by MSCs reportedly involves various growth factors and cytokines [16], and several studies have shown that HGF attenuates CKD symptoms. Specifically, injections with HGF attenuated the progression of glomerulosclerosis and proteinuria in 5/6-nephrectomized rats or nephrotic mice [3] [6]. Moreover, implanted MSCs ameliorated glomerular injuries by secreting HGF in a rat model of diabetic nephropathy [17]. The biological effects of HGF include the enhancement of cell growth, anti-apoptotic and angiogenic activity [18], and renoprotective effects [19]. In the present *in vitro* experiments, HGF production was enhanced in MSCs captured on nonwoven PLA filters, and these cells improved glomerulosclerosis after implantation *in vivo*. In contrast, a topical application of nonwoven PLA filters without cells, or of r-BMSCs alone, onto kidney surfaces did not significantly improve or prevent glomerulosclerosis. These data suggest that glomerulosclerosis was ameliorated by enhanced HGF production.

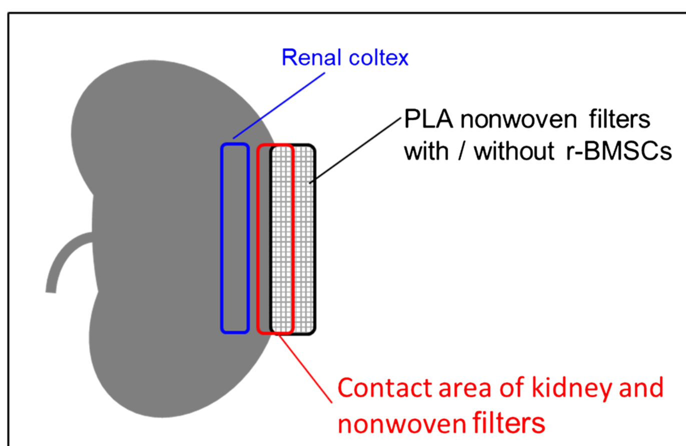
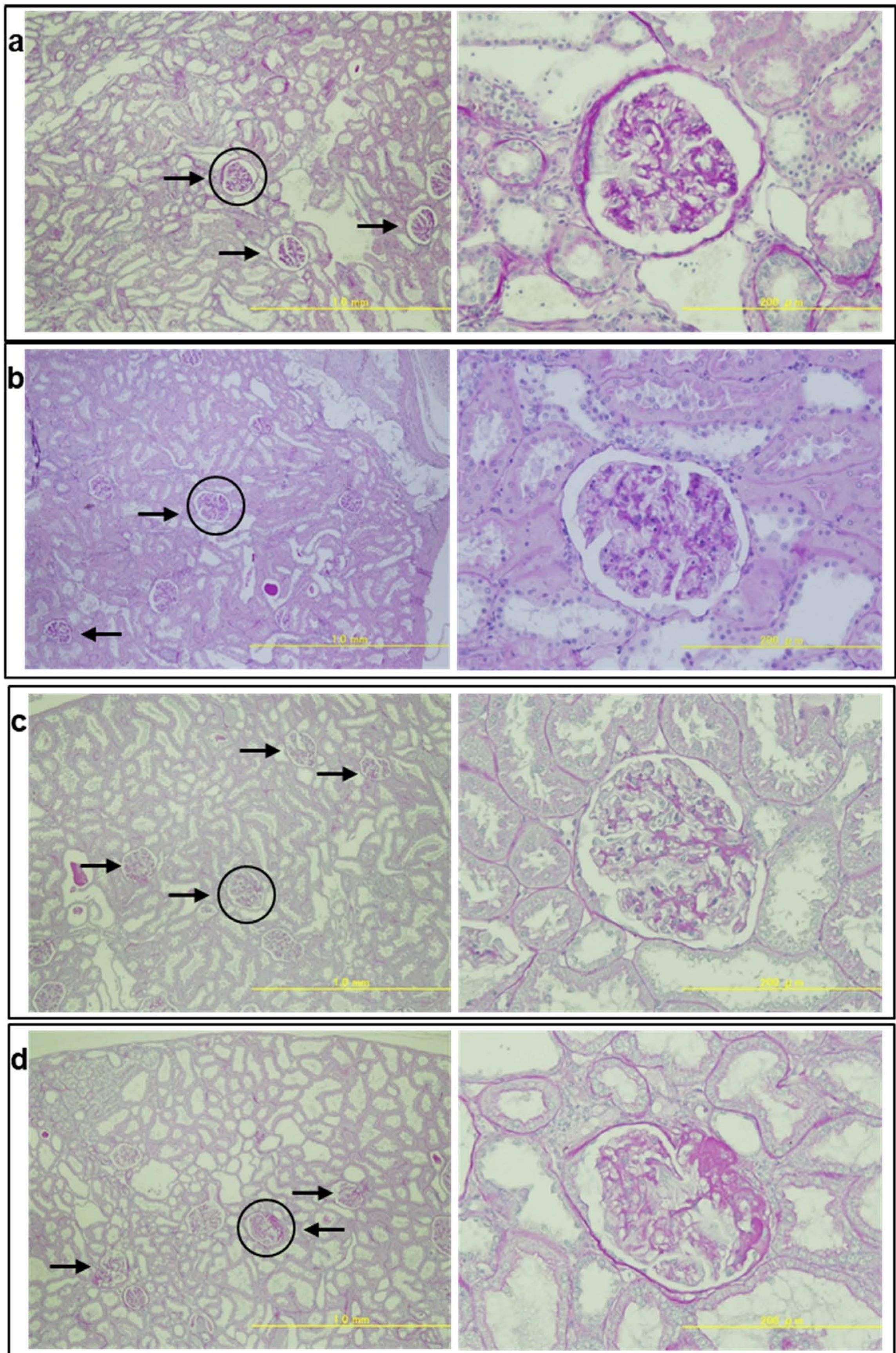


Figure 4. Schema of the contact area of the kidney and nonwoven filter.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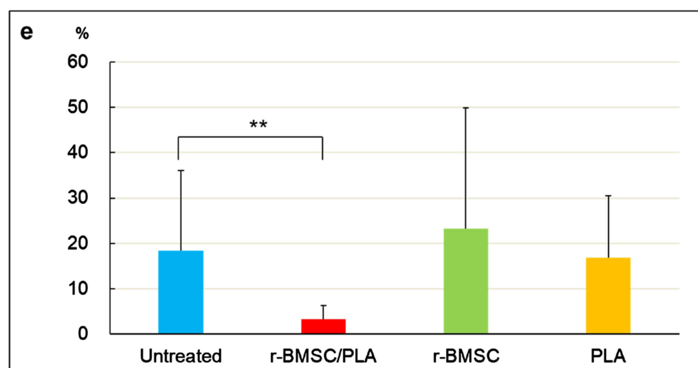

Figure 5. Histopathological evaluations of kidneys after treatment using nonwoven filters with captured cells (PAS). (a) Untreated 5/6-nephrectomized rats. (b) 5/6-nephrectomized rats treated with r-BMSCs on five PLA filter disks. (c) 5/6-nephrectomized rats treated with r-BMSCs. (d) 5/6-nephrectomized rats treated with five PLA filter disks. Black arrows indicate glomerulosclerotic lesions. The right column is an enlarged image of the glomerulus shown in the circle of the left column. Yellow scale bars in bottom right corner of images are 1.0 mm (left column) and 200 μm (right column) insets. (e) Incidence of glomerulosclerotic lesions. Untreated (n = 10), untreated 5/6-nephrectomized rats; r-BMSC/PLA (n = 6), 5/6-nephrectomized rats treated with r-BMSCs on five PLA filter disks; r-BMSC (n = 4), 5/6-nephrectomized rats treated with r-BMSCs; PLA (n = 4), 5/6-nephrectomized rats treated with five PLA filter disks; $^{**}p < 0.01$ vs. untreated group.

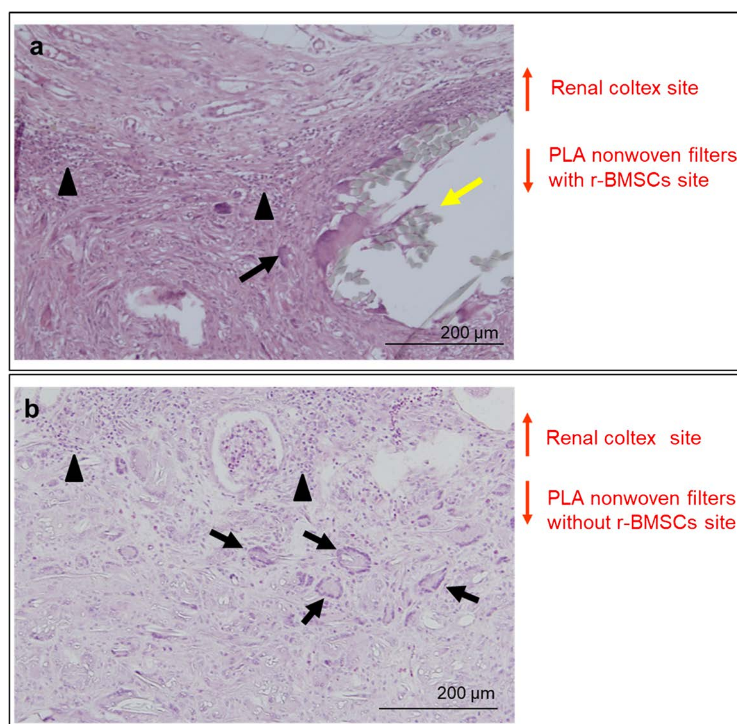

Figure 6. Histopathology of the contact area of kidney and PLA filters 8 weeks after implantation with PLA nonwoven filters with or without cells. (H & E). (a) Pathological photomicrograph of the contact area between the PLA filters with r-BMSCs and the kidney of 5/6-nephrectomized rats. (b) Pathological photomicrograph of the contact area between the PLA filters without r-BMSCs and the kidney of 5/6-nephrectomized rats. Black arrows indicate multinucleated foreign body giant cells; black arrowheads indicate lymphocytes; yellow arrow indicates decomposed matter of PLA filter.

Moreover, PLA filters suppressed VEGF production during the first 3 weeks of culture, whereas VEGF production in the presence of PLA filters with r-BMSCs was similar to that in control groups at 4 weeks. Because the production of VEGF in PLA filters with r-BMSCs gradually increased during the 4-week culture period, the production of VEGF may have been enhanced after culture for longer than 4 weeks. These data further suggest that growth factors such as HGF and VEGF were secreted from r-BMSCs fixed on PLA filters, and that they may contribute to restorative paracrine signaling in wounded glomeruli. When the r-BMSC/PLA complex was implanted after 4 weeks of culture, the subsequent augmented production of both HGF and VEGF was expected to have a major therapeutic effect.

The implantation of r-BMSC/PLA into 5/6-nephrectomized rats attenuated urinary protein excretion after 4 weeks. However, at 8 weeks after implantation, this effect of decreasing urinary protein disappeared. There are two potential reasons behind this development. The PLA nonwoven filter may have been fragmented due to a foreign matter reaction by polynuclear foreign body giant cells; the nonwoven filter indeed appeared to be highly decomposed at 8 weeks after implantation. Moreover, the degree of decomposition did not change depending on the presence or absence of r-BMSCs. Inflammatory reactions are mainly caused by biomaterials embedded in the body, or the degradation of these products [20]. Furthermore, in the cell interaction process during the chronic phase, polynuclear foreign body giant cells are formed, and the formed multinuclear foreign body giant cells degrade biodegradable substances over time [21]. Inflammation is also essential to promote the recruitment of progenitor cells and to initiate healing mechanisms, but at the same time results in tissue damage and fiber inclusion of biocompatible substances [22]. In this study, we did not investigate the presence of r-BMSCs around the implantation site, but polynuclear foreign body giant cells and lymphocytes were observed, suggesting the possibility that fibrosis was initiated in the nonwoven filters. This is a possible reason why urine protein concentrations did not decrease at 8 weeks after implantation. Another possible reason is that the r-BMSCs captured on the PLA nonwoven filter could not adhere to a sufficient area of the damaged kidney surface. Indeed, in some rats it was observed that the r-BMSCs captured on PLA nonwoven filters were partially separated from the kidney surface (data not shown). Therefore, a nonwoven filter affixing method covering a sufficient area of the injured kidney surface, as well as an implantation method allowing the complex of cells and nonwoven filters to exist on the kidney surface for a longer period, requires future study.

5. Conclusion

Here, we report the development of a novel therapeutic device made up of biodegradable PLA filters and r-BMSCs. After implantation into 5/6-nephrectomized rats, these devices reduced urinary protein levels and prevented or ameliorated

glomerulosclerosis. These effects might be related to enhanced HGF production by r-BMSCs that were captured on the PLA filters. This device requires further consideration as a treatment for CKD.

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Conflicts of Interest

All authors declare that they have no conflict of interest.

References

[1] Liyanage, T., Ninomiya, T., Jha, V., Neal, B., Patrice, H.M., Okpechi, I., *et al.* (2015) Worldwide Access to Treatment for End-Stage Kidney Disease: A Systematic Review. *The Lancet*, **385**, 1975-1982. https://doi.org/10.1016/S0140-6736(14)61601-9

[2] Just, P.M., Riella, M.C., Tschosik, E.A., Noe, L.L., Bhattacharyya, S.K. and de Charro, F. (2008) Economic Evaluations of Dialysis Treatment Modalities. *Health Policy*, **86**, 163-180. https://doi.org/10.1016/j.healthpol.2007.12.004

[3] Mizuno, S., Kurosawa, T., Matsumoto, K., Mizuno-Horikawa, Y., Okamoto, M. and Nakamura, T. (1998) Hepatocyte Growth Factor Prevents Renal Fibrosis and Dysfunction in a Mouse Model of Chronic Renal Disease. *The Journal of Clinical Investigation*, **101**, 1827-1834. https://doi.org/10.1172/JCI1709

[4] Flaquer, M., Romagnani, P. and Cruzado, J.M. (2010) Growth Factors and Renal Regeneration. *Nefrologia*, **30**, 385-393.

[5] Shimizu, A., Masuda, Y., Mori, T., Kitamura, H., Ishizaki, M., Sugisaki, Y., *et al.* (2004) Vascular Endothelial Growth Factor165 Resolves Glomerular Inflammation and Accelerates Glomerular Capillary Repair in Rat Anti-Glomerular Basement Membrane Glomerulonephritis. *Journal of the American Society of Nephrology*, **15**, 2655-2665. https://doi.org/10.1097/01.ASN.0000141038.28733.F2

[6] Gong, R., Rifai, A., Tolbert, E.M., Biswas, P., Centracchio, J.N. and Dworkin, L.D. (2004) Hepatocyte Growth Factor Ameliorates Renal Interstitial Inflammation in Rat Remnant Kidney by Modulating Tubular Expression of Macrophage Chemoattractant Protein-1 and RANTES. *Journal of the American Society of Nephrology*, **15**, 2868-2881. https://doi.org/10.1097/01.ASN.0000141962.44300.3A

[7] Yang, J., Chen, S., Huang, L., Michalopoulos, G.K. and Liu, Y. (2001) Sustained Expression of Naked Plasmid DNA Encoding Hepatocyte Growth Factor in Mice Promotes Liver and Overall Body Growth. *Hepatology*, **33**, 848-859. https://doi.org/10.1053/jhep.2001.23438

[8] Ezquer, F., Ezquer, M., Simon, V., Pardo, F., Yañez, A., Carpio, D., *et al.* (2009) Endovenous Administration of Bone-Marrow-Derived Multipotent Mesenchymal Stromal Cells Prevents Renal Failure in Diabetic Mice. *Biology of Blood and Marrow Transplantation*, **15**, 1354-1365. https://doi.org/10.1016/j.bbmt.2009.07.022

[9] Zoja, C., Garcia, P.B., Rota, C., Conti, S., Gagliardini, E., Corna, D., *et al.* (2012)

Mesenchymal Stem Cell Therapy Promotes Renal Repair by Limiting Glomerular Podocyte and Progenitor Cell Dysfunction in Adriamycin-Induced Nephropathy. *American Journal of Physiology—Renal Physiology*, **303**, F1370-F1381. https://doi.org/10.1152/ajprenal.00057.2012

[10] Semedo, P., Correa-Costa, M., Cenedeze, M.A., Malheiros, D.M.A.C., dos Reis, M.A. and Shimizu M.H. (2009) Mesenchymal Stem Cells Attenuate Renal Fibrosis through Immune Modulation and Remodeling Properties in a Rat Remnant Kidney Model. *Stem Cells*, **27**, 3063-3073. https://doi.org/10.1002/stem.214

[11] Song, I.H., Jung, K.J., Lee, T.J., Kim, J.Y., Sung, E.G., Bae, Y.C., *et al.* (2018) Mesenchymal Stem Cells Attenuate Adriamycin-Induced Nephropathy by Diminishing Oxidative Stress and Inflammation via Downregulation of the NF-kB. *Nephrology*, **23**, 483-492. https://doi.org/10.1111/nep.13047

[12] Schrepfer, S., Deuse, T., Reichenspurner, H., Fischbein, M.P., Robbins, R.C. and Pelletier, M.P. (2007) Stem Cell Transplantation: The Lung Barrier. *Transplantation Proceedings*, **39**, 573-576. https://doi.org/10.1016/j.transproceed.2006.12.019

[13] Hori, H., Iwamoto, U., Niimi, G., Shinzato, M., Hiki, Y., Tokushima, Y., *et al.* (2015) Appropriate Nonwoven Filters Effectively Capture Human Peripheral Blood Cells and Mesenchymal Stem cells, Which Show Enhanced Production of Growth Factors. *Journal of Artificial Organs*, **18**, 55-63. https://doi.org/10.1007/s10047-014-0794-9

[14] Iwamoto, U., Hori, H., Takami, Y., Tokushima, Y., Shinzato, M., Yasutake, M., *et al.* (2015) A Novel Cell-Containing Device for Regenerative Medicine: Biodegradable Nonwoven Filters with Peripheral Blood Cells Promote Wound Healing. *Journal of Artificial Organs*, **18**, 315-321. https://doi.org/10.1007/s10047-015-0845-x

[15] Shimamura, T. and Morrison, A.B. (1975) A Progressive Glomerulosclerosis Occurring in Partial Five-Sixths Nephrectomized Rats. *The American Journal of Pathology*, **79**, 95-106. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1913032/

[16] Caplan, A.I. and Dennis, J.E. (2006) Mesenchymal Stem Cells as Trophic Mediators. *Journal of Cellular Biochemistry*, **98**, 1076-1084. https://doi.org/10.1002/jcb.20886

[17] Lv, S., Cheng, J., Sun, A., Li, J., Wang, W., Guan, G., *et al.* (2014) Mesenchymal Stem Cells Transplantation Ameliorates Glomerular Injury in Streptozotocin-Induced Diabetic Nephropathy in Rats via Inhibiting Oxidative Stress. *Diabetes Research and Clinical Practice*, **104**, 143-154. https://doi.org/10.1016/j.diabres.2014.01.011

[18] Matsumoto, K. and Nakamura, T. (2001) Hepatocyte Growth Factor: Renotropic Role and Potential Therapeutics for Renal Diseases. *Kidney International*, **59**, 2023-2038. https://doi.org/10.1046/j.1523-1755.2001.00717.x

[19] Cantaluppi, V., Biancone, L., Quercia, A., Deregibus, M.C., Segoloni, G. and Camussi, G. (2013) Rationale of Mesenchymal Stem Cell Therapy in Kidney Injury. *American Journal of Kidney Diseases*, **61**, 300-309. https://doi.org/10.1053/j.ajkd.2012.05.027

[20] Ding, J., Chen, B., Lv, T., Liu, X., Fu, X., Wang, Q., *et al.* (2016) Bone Marrow Mesenchymal Stem Cell-Based Engineered Cartilage Ameliorates Polyglycolic Acid/Polylactic Acid Scaffold-Induced Inflammation through M2 Polarization of Macrophages in a Pig Model. *Stem Cells Translational Medicine*, **5**, 1079-1089. https://doi.org/10.5966/sctm.2015-0263

[21] Ramot, Y., Haim-Zada, M., Domb, A.J. and Nyska, A. (2016) Biocompatibility and Safety of PLA and Its Copolymers. *Advanced Drug Delivery Reviews*, **107**, 153-162. https://doi.org/10.1016/j.addr.2016.03.012

[22] McNally, A.K. and Anderson, J.M. (2003) Foreign Body-Type Multinucleated Giant

Cell Formation Is Potently Induced by Alpha-Tocopherol AND Prevented by the Diacylglycerol Kinase Inhibitor R59022. *The American Journal of Pathology*, **163**, 1147-1156. https://doi.org/10.1016/S0002-9440(10)63474-8