In Vivo Safety and Regeneration of Long-Term Transported Amniotic Fluid Stem Cells for Renal Regeneration

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Abstract

BACKGROUND: Despite major progress in stem cell therapy, our knowledge of the characteristics and tissue regeneration potency of long-term transported cells is insufficient. In a previous *in vitro* study, we established the optimal cell transport conditions for amniotic fluid stem cells (AFSCs). In the present study, the target tissue regeneration of long-term transported cells was validated *in vivo*.

METHODS: For renal regeneration, transported AFSCs were seeded on a poly(lactide-co-glycolide) scaffold and implanted in a partially resected kidney. The target tissue regeneration of the transported cells was compared with that of freshly harvested cells in terms of morphological reconstruction, histological microstructure reformation, immune cell infiltration, presence of induced cells, migration into remote organs, expression of inflammation/fibrosis/renal differentiation-related factors, and functional recovery.

RESULTS: The kidney implanted with transported cells showed recovery of total kidney volume, regeneration of glomerular/renal tubules, low CD4/CD8 infiltration, and no occurrence of cancer during 40 weeks of observation. The AFSCs gradually disappeared and did not migrate into the liver, lung, or spleen. We observed low expression levels of pro-inflammatory cytokines and fibrotic factors; enhanced expression of the genes *Wnt4*, *Pax2*, *Wt1*, and *Emx2*; and significantly reduced blood urea nitrogen and creatinine values. There were no statistical differences between the performance of freshly harvested cells and that of the transported cells.

CONCLUSION: This study demonstrates that long-term transported cells under optimized conditions can be used for cell therapy without adverse effects on stem cell characteristics, *in vivo* safety, and tissue regeneration potency.

Keywords Amniotic fluid stem cell · Long-term cell transportation · Tissue regeneration

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1 Introduction

Cell therapies have been used since the 1960s, when the first bone marrow transplants were conducted [1]. The range of cell therapies is likely to increase in the future as

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researchers explore the potential of stem cells to treat a wide range of illnesses. However, one of the major challenges faced by the rapidly growing number of stem cell therapies is the transport of fragile living cells without damaging their viability, characteristics, and potency. If patients use autologous stem cells and their manufacture occurs in or close to the hospital, these concerns are reduced because freshly harvested cells can be used quickly. However, if the cells are manufactured far from the hospital and the patients use allogenic stem cells, such as amniotic fluid stem cells (AFSCs), the time from manufacture to application will be longer. Moreover, the cells must be transported for several hours, during which their viability might decrease or their characteristics might be altered [2], reducing their target tissue regenerative potency.

To minimize these problems, in a previous study we optimized the cell transport conditions [3] through an *in vitro* analysis of the medium, temperature, cell density, and container type. For AFSCs, we suggested an appropriate cell suspension medium comprising high-glucose Dulbecco's modified minimal Eagle's medium (DMEM), a transport temperature of 4 °C, a cell density of $1 \times 10^7/2$ mL, and a disposable syringe as the container. Cells transported under these conditions maintained more than 80% survival and stem cell characteristics for 12 h [3]. These cells would be ready to use in the clinical field without additional treatment, such as removal of toxic cryoprotectants, and complex cell thawing steps to prevent osmotic shock.

However, knowledge of the *in vivo* response of long-term transported cells is very limited. *In vitro* experiments can provide a great deal of information. But this information is not complete until verified by examining the *in vivo* responses of cells. The main mechanism of stem cells in tissue regeneration is the paracrine effect. The types of secreted molecules and the duration of their activities are different according to the surrounding *in vivo* environment. Rational environments to consider include the type of traumatic injury, oxygen free radical status, immune cell activity, type of extracellular matrix, endogenous stem cell

populations, exposure to inflammatory/fibrosis cytokines, biophysical conditions, and induction of thermal shock [4].

In this study, the *in vivo* response of long-term transported cells was assessed through renal regeneration. The kidney is one of the major target organs in regenerative medicine. Presently, we established histological, functional, and molecular methods to evaluate renal regeneration. To minimize other variables, except for the cell status, these established techniques included partial nephrectomy and poly(lactide-co-glycolide) (PLGA) scaffold combination [5, 6]. Therefore, we verified the long-term transported AFSCs' *in vivo* safety, inflammation, fibrosis, functional recovery, and renal tissue regeneration ability, with freshly harvested cells used as a control.

2 Materials and methods

2.1 Preparation of human AFSCs

This study was approved by the Ethics Committee of Kyungpook National University School of Medicine. Amniotic fluid (10 mL) was obtained by informed written consent from a woman undergoing routine amniocentesis (16–20 weeks gestational age) at Kyungpook National University Hospital. AFSCs were cultured in Chang Medium containing 15% fetal bovine serum (Gibco, Grand Island, NY, USA), 1% L-glutamine, and 1% penicillin/streptomycin with 18% Chang B and 2% Chang C (Irvine Scientific, Irvine, CA, USA) at 37 °C in an atmosphere of 5% CO₂. Three days later, debris and non-adherent cells were discarded and the medium was replaced by fresh Chang medium. Attached AFSCs were expanded and passaged at 80% confluence. Cells at passage 5 were used for subsequent experiments.

2.2 AFSC transportation conditions

According to the established transport conditions, 1×10^7 AFSCs were suspended in 2 mL of high-glucose DMEM [DMEM(H)], contained in a moving box kept at 4 °C, and transported for 12 h. Control cells were prepared immediately after harvest.

2.3 Scaffold preparation

A PLGA/Mg(OH)₂ scaffold was fabricated using a freeze-drying method. Ice microparticles (200–300 µm) were prepared by spraying cold deionized water into liquid nitrogen. A 13% (w/v) solution of PLGA (MW = 40 kDa; Boehringer Ingelheim, Ingelheim am Rhein, Germany) in methylene chloride was mixed with 0.15 g of Mg(OH)₂ (Sigma-Aldrich, St Louis, MO, USA) nanoparticles, which

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were synthesized by reacting 5 g of $\text{Mg}(\text{NO}_3)_2$ and 1.6 g of NaOH. The solution was vortexed and the ice microparticles were added to the pre-cooled PLGA solution. The mixture was frozen in a silicon mold in liquid nitrogen and then freeze-dried for 2 days to form the PLGA/ $\text{Mg}(\text{OH})_2$ scaffold. The scaffold was sterilized with 70% ethanol for 30 min and rinsed three times with phosphate-buffered saline (PBS; pH 7.4) for 10 min.

2.4 In vivo experimental design

All experimental protocols were approved by the Yeungnam University Institutional Animal Care and Use Committee. Five-week-old ICR (male) mice (average body weight, 20 g) were obtained from Central Lab. Animal Inc. (Seoul, Korea). Sixty mice were randomly divided into four groups ($n = 15$ in each group): (1) Control (Ctrl) group, sham operated; (2) PLGA group, PLGA scaffold implanted without cells; (3) Fresh cell group, freshly harvested cells seeded on the PLGA scaffold; and (4) Transport cell group, 12 h transported cells seeded on the PLGA scaffold. The scaffold volume was $5 \times 2 \times 2 \text{ mm}^3$, and the number of cells per scaffold was 1×10^6 . The right kidney was exposed through an incision on the back, and a partial nephrectomy was performed (an injured region was made on the renal cortex, with an excision volume of $5 \times 2 \times 2 \text{ mm}^3$). The cells were seeded on the scaffold 30 min before implantation. The cell-scaffold complexes were implanted at the injured region and the left kidney was removed.

2.5 Analysis of renal function

Renal function was assessed at 1, 4, and 8 weeks after implantation using serum blood urea nitrogen (BUN) and creatinine levels. One milliliter of blood was obtained from the heart without anticoagulant treatment, and placed at room temperature for 15 min. The collected serum was stored at 4°C until analysis. Commercial kits were used to measure BUN (Arbor assays, Ann Arbor, MI, USA) and creatinine (Abcam, Cambridge, UK) values.

2.6 Histological, immunohistochemical (IHC), and real-time PCR analyses

Kidneys were retrieved at 1, 4, and 8 weeks after surgery ($n = 5$ for each time point) and the renal weight was measured. Specimens were fixed in 4% paraformaldehyde and paraffin-embedded samples were cut into $5\text{-}\mu\text{m}$ sections. For histological analysis, the sections were stained with hematoxylin and eosin (H&E). The migrated host stem cells were averaged by counting 10 images at $400 \times$ magnification, without overlap at week 1. The

number of regenerated glomeruli per unit area ($4860 \mu\text{m}^2$) was counted at week 8.

In the IHC analysis, immune cell infiltration was detected using anti-CD4 and CD8 antibodies. The presence and duration of the implanted cells were determined using a human nuclear-specific (HuNu) antibody. Migration into distant organs was detected using the HuNu antibody on liver, lung, and spleen samples. The details of antibodies used for IHC are provided in Table 1.

For real-time PCR, total RNA was isolated from each group using Trizol reagent (Thermo Fisher Scientific, Waltham, MA, USA) and cDNA was synthesized from $1 \mu\text{g}$ of RNA using a reverse transcription kit (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed using the ABI Prism Sequence Detection System 7500 (Applied Biosystems) for all genes. Individual PCRs were carried out in $10 \mu\text{L}$ volumes containing $2 \mu\text{L}$ of DEPC water, $1 \mu\text{L}$ of forward and reverse primers (10 pM), and $5 \mu\text{L}$ SYBR Green PCR Master Mix (Applied Biosystems). The reaction conditions comprised 10 min of pre-incubation at 95°C , followed by 45 cycles for 10 s at 95°C , 50 s at 58°C , and 20 s at 72°C . *ACTB* (encoding beta-actin) was used as a control gene and the $2^{-\Delta\Delta\text{Ct}}$ method was adapted to estimate copy numbers. For more accurate analysis of the administered human cells, a human specific beta globin primer was used. For this, genomic DNA was obtained using a Wizard® Genomic DNA Purification Kit (Promega, Fitchburg, WI, USA) according to the manufacturer's instructions. Primer information is provided in Table 2.

2.7 Statistical analysis

Data are expressed as the mean $\pm$ standard deviation (SD). Statistically significant differences were determined by a *t*-test and one-way analysis of variance with Tukey's post hoc test. Differences were considered significant at a *P* value < 0.05 .

Table 1 Antibodies used in immunohistochemistry for human cell tracking and inflammation analysis

Antibody	Company	Dilution
<i>Primary antibody</i>		
CD4	BD Pharmingen	1:200
CD8	Biorbyt	1:200
Human nuclei (HuNu)	Millipore	1:200
<i>Secondary antibody</i>		
Alexa Fluor 594 Goat anti-rabbit IgG	Thermo	1:1000
Alexa Fluor 594 Goat anti-mouse IgG	Thermo	1:1000

Table 2 Real-time PCR primer sequences

Gene	Sequences
<i>β-actin</i>	5'- AGCCATGTACGTAGCCATCC 5'- CTCTCAGCTGTGGTGGTGAA
<i>IL-10</i>	5'- ATAAGTGCACCCACTTCCCA 5'- AGGGTCTTCAGCTTCTCACC
<i>IL-4</i>	5'- TCAACCCCCAGCTAGTTGTC 5'- TGTTCTTCGTTGCTGTGAGG
<i>IL-2</i>	5'- CCCACTTCAAGCTCCACTTC 5'- ATCCTGGGGAGTTTCAGGTT
<i>TGF-β1</i>	5'- TTGCTTCAGCTCCACAGAGA 5'- TGGTTGTAGAGGGCAAGGAC
<i>TNF-α</i>	5'- AGCCCCCAGTCTGTATCCTT 5'- CTCCCTTTCAGAACTCAGG
<i>IL-6</i>	5'- AGTTGCCTTCTTGGGACTGA 5'- TCCACGATTTCCAGAGAAC
<i>IL-1β</i>	5'- GCCCATCCTCTGTGACTCAT 5'- AGGCCACAGGTATTTTGTCTG
<i>Vimentin</i>	5'- TCCAGATCGATGTGGACGTT 5'- ATACTGCTGGCGCACATCAC
<i>Coll</i>	5'- GAGCGGAGAGTACTGGATCG 5'- GCTTCTTTTCCTTGGGGTTC
<i>α-SMA</i>	5'- CTGACAGAGGCACCACTGAA 5'- CATCTCCAGAGTCCAGCACA
<i>Wnt</i>	5'- GCAGCCGTCAATGGCTTTAG 5'- GGAGCCTAGCCCGGACTTC
<i>Pax2</i>	5'- AAATCTCTATGCAAAATGACGAACAT 5'- GAGAGATGCAGGGCGATGAA
<i>Wt1</i>	5'- GGTCCGCCATCACAAACATG 5'- CTTTCCTGCTGGGATGCT
<i>Emx2</i>	5'- TGGCCAGAAAGCCAAAGC 5'- TCCGCTCCCACCACGTAAT
<i>vWF</i>	5'- GCTGTGCGGTGATTTTAACATC 5'- CCGTTTACACCGCTGTTCTT
<i>hβ-globin</i>	5'- GTGCACCTGACTCCTGAGGAGA3 5'- CCTTGATACCAACCTGCCCAGG

3 Results

3.1 Comparison of gross morphology

Under gross examination (Fig. 1A), the kidneys implanted with scaffolds showed swelling at week 1, which gradually reduced until week 4, and which were not visible at week 8 (yellow dotted line). The experimental kidneys showed faint scars at the scaffold-implanted site at week 8. The removed parts of the experimental kidneys were refilled

Fig. 1 Comparison of the morphology and histology between kidneys implanted with freshly harvested cells and transported cells. **A** Gross morphology of experimental kidneys. The dotted line represents the site of scaffold implantation. **B** Hematoxylin and eosin (H&E) staining for histological comparison and *in vivo* safety assessment. **C** Number of migrated host cells at week 1. **D** Number of regenerated glomeruli per unit area ($4860 \mu\text{m}^2$) at week 8. #: An empty space in which the scaffold is degraded; thin arrow, infiltrated host cell; thick black arrow, newly formed glomerulus; and thick white arrow, newly formed renal tubules. Control, sham-operated normal kidney; PLGA, kidney implanted with a poly(lactide-co-glycolide) (PLGA) scaffold (without cells); Fresh cell, kidney implanted with freshly harvested cells seeded on a PLGA scaffold; Transport cell, a kidney implanted with transported cells (under optimized conditions) seeded on a PLGA scaffold. * $P < 0.05$ and ** $P < 0.01$; $n = 5$ for each group scale bar $20 \mu\text{m}$. (Color figure online)

without statistically significant change in renal weight ($0.27 \pm 0.02 \text{ g}$) compared with the control ($0.26 \pm 0.08 \text{ g}$) at week 8. No morphological differences were found between the fresh cell and transported cell implanted groups.

3.2 Comparison of structural regeneration potency and *in vivo* safety

Scaffold degradation, immune cell infiltration, and renal tissue regeneration were analyzed by H&E staining (Fig. 1B–D). At week 1 (Fig. 1B–a–c), the scaffolds were degraded and showed empty spaces (marked with #), PLGA degradation debris was localized in adjacent renal tissues, immune cells appeared around the scaffold, and renal-related cells were located at the scaffold-diminished site (arrow). The numbers of migrated host cells were similar in the fresh cell and transport cell groups (Fig. 1C). At week 4, the injured regions of both cell-seeded groups were filled with renal cells, renal tubules were regenerating (white arrow), and numerous premature glomeruli were observed (black arrow) (Fig. 1B–e, f). Newly formed glomerular numbers in the fresh cell and transport cell groups were similar (Fig. 1D), and this number was almost the same as that in the control group. The PLGA group showed severe immune cell infiltration and did not form premature glomeruli or tubular structures (Fig. 1B–b–d). At week 8, the scaffolds in both cell-seeded groups were not detected, renal tubule structures were nearly regenerated, and mature glomeruli were apparent in the regenerated region (Fig. 1B–h, i). In the PLGA group, the scaffold-implanted area showed markedly reduced formation of glomeruli and renal tubules, and wider fibrotic morphology in the regenerated region (Fig. 1B–g). During the experimental period, no groups showed abnormal tissue formation.

3.3 Comparison of immune responses, cell survival, and migration

For the IHC analysis, infiltration of cytotoxic and helper T cells into the scaffold implanted region was detected using anti-CD4 and CD8 antibodies, respectively (Fig. 2A, B). At week 1, both the fresh and transported cell groups showed similarly low numbers of CD4- and CD8-positive cells (red) compared with those in the PLGA group. At week 4, the numbers of CD4 and CD8 positive cells were reduced further. HuNu antibody was used to identify induced human cells; both the fresh and transported cell groups showed significantly positive HuNu reactivity at

week 1, but not at week 4 and 8 (Fig. 2C). No migration of implanted human cells into distant organs was observed in the liver, lung, and spleen at weeks 1, 4, and 8 (Fig. 2D). PCR analysis using the human beta globin gene showed that the human cells were found only in the kidney at 1 week, and not in other surrounding tissues (Fig. 2E).

3.4 Comparison of inflammatory and fibrotic responses

Real-time PCR analysis for the expression of anti-inflammatory factor genes (Fig. 3A) showed that the fresh and transported cell groups had relatively high expression of

Fig. 2 Immunohistochemical analysis of kidneys implanted with freshly harvested cells or transported cells. **A** Cytotoxic T cells identified using an anti-CD4 antibody. **B** Helper T cells identified using an anti-CD8 antibody. **C** Human amniotic fluid stem cells (AFSCs) tracked using the human nuclear-specific (HuNu) antibody. **D** Human AFSC migration into distant organs tracked using the HuNu antibody. **E** Identification of human β -globin to quantitatively demonstrate that the introduced human cells did not migrate to other

distant organs. Arrow: Cells positive for each antibody. Control, sham operated normal kidney; PLGA, kidney implanted with a poly(lactide-co-glycolide) (PLGA) scaffold (without cells); Fresh cell, kidney implanted with freshly harvested cells seeded on a PLGA scaffold; Transport cell, a kidney implanted with transported cells (under optimized conditions) seeded on a PLGA scaffold. $n = 5$ for each group scale bar 20 μ m

Fig. 2 continued

these genes compared with that in the control. In particular, the transported cell group showed significantly higher expression of *Tgfb1* at week 1 and 4 compared with that in the fresh cell group. For pro-inflammatory (Fig. 3B) and fibrosis (Fig. 3C) factors, the fresh and transported cell groups showed high expression at early time points, which gradually decreased to normal values. Although *Il1b* and *Col1* expression levels increased in the transported cell group at week 8, the difference between the two cell types was not significant.

3.5 Comparison of renal differentiation gene expression and functional recovery

In the renal differentiation genes expression analysis, the transported cell group showed slightly, but not significantly high, expression levels compared with the fresh cell group (Fig. 4A). At the early time points (1 and 4 weeks), *Wnt4*,

Pax2, *Wt1*, and *Emx2* gene expressions were substantially higher in the transported cell group than in the fresh cell group.

The functions of the regenerated kidneys were compared using serum BUN and creatinine values (Fig. 4B). At week 1, the BUN concentrations in the control, PLGA, fresh, and transported cell groups were 28.6 ± 0.27 , 38.8 ± 2.1 , 31.5 ± 3.72 , and 31.7 ± 0.85 mg/dL, and the creatinine levels were 0.27 ± 0.003 , 0.56 ± 0.09 , 0.305 ± 0.007 , and 0.306 ± 0.005 mg/dL, respectively. The BUN and creatinine values of the fresh and transported cell groups were significantly lower than those of the PLGA group, and there was no difference between the values for the fresh and transport cell groups. At weeks 4 and 8, the serum BUN and creatinine levels of the fresh and transported cell groups were also lower than those of the control, and there was no difference between the values for the fresh and transported cell groups.

Fig. 2 continued

4 Discussion

When a partial nephrectomy is performed, the total renal volume is reduced and glomerular and renal tubules are damaged, which results in decreased renal function. Cell therapy might regenerate the glomeruli and renal tubules, allowing recovery of renal function to the normal level. In this study, we chose AFSCs as the cell source because AFSCs have many benefits for clinical applications. Perin et al. demonstrated that injected AFSCs exert a protective effect by ameliorating acute tubular necrosis, as reflected by decreased BUN and creatinine levels, and reduced numbers of damaged tubules and apoptosis. AFSCs also showed immunomodulatory effects [7]. These observations suggest that AFSCs have an intrinsic ability to differentiate into renal cell types, and potentially represent a limitless and ethically neutral cell source [8]. The regenerative effect of AFSCs in the damaged kidney is mediated

through local paracrine action, likely to be accomplished by the secretion of cytokines and growth factors, including vascular endothelial growth factor, stromal cell-derived factor-1 (SDF-1), and insulin-like growth factor-1 (IGF-1) [9, 10]. In our study, AFSCs transported for 12 h maintained their stem cell characteristics, similar to those of freshly harvested cells. This makes them useful for structural and functional renal regeneration by stimulating the migration and differentiation of surrounding cells and suppressing the initial inflammatory response.

The scaffolds comprising the freshly harvested and transported cells were gradually degraded, the implanted site was replaced with renal tissue, and the total kidney weight recovered to the control level. Histological analysis with H&E staining showed immune cell infiltration in response to the acidic degraded product from the PLGA polymer [11] at week 1, but both cell-seeded groups effectively suppressed immune cell infiltration compared

Fig. 2 continued

with the PLGA group, and there was no difference in the number of immune cells between the two cell groups. The migration pattern of the surrounding donor cells into the empty space was similar in both cell groups. In addition, the size and number of regenerated glomeruli and renal tubules were similar in both cell groups.

In the IHC analysis, the number of CD4 and CD8 positive cells were similarly low in both cell groups, indicating that the transported cells maintained their ability to secrete anti-inflammatory cytokines [12]. HuNu-positive cells were detected at week 1, but not at week 4 and 8 in both cell groups. In addition, the freshly harvested and transported cells did not migrate into other organs as do mesenchymal stem cells (MSCs), indicating that the

transported cells were safe to use in the body. According to a previous study, induced MSCs in the body could be detected histologically for 2 weeks [13]. *In vivo* cell migration patterns differ between embryonic stem cells (ESCs) and MSCs. In animal studies, MSCs were located only at the administered site, while ESCs could migrate to remote organs [14]. AFSCs have both ESC and MSC characteristics, thus it is necessary to analyze whether the cells migrate to distant organs from injection site. The target remote organs were the lung, spleen, and liver, because injected ESCs were reported to migrate mainly into these organs [15]. Entrapment of stem cells in the remote organs may have adverse consequences [16], such as embolism in the lungs, and osteosarcoma caused by

Fig. 3 Comparison of inflammation and fibrosis related gene expression between freshly harvested cells and transported cells. **A** Expression of anti-inflammatory factors. **B** Expression of pro-inflammatory factors. **C** Expression of fibrotic factors. Ct: sham operated normal kidney; PL: implanted with poly(lactide-co-glycolide) (PLGA)

scaffold (without cells); F: implanted with freshly harvested cells seeded on a PLGA scaffold; T: implanted with transported cells (under optimized conditions) seeded on a PLGA scaffold. * $P < 0.05$ and ** $P < 0.01$; $n = 5$ for each group

osteogenic differentiation and calcium deposits within the capillaries [17]. These influences are mainly detected in the lung, because the capillary size and surface properties can promote stem cell attachment [16].

Allo-MSCs can stimulate innate immune responses [18]. Introducing the cells into the body stimulated the expression of anti-inflammatory cytokines in the surrounding tissue environment. The expression levels of *Il10* and *Il4* gradually increased over time, and *Il2* and *Tgfb1* expression increased up to week 4 and then decreased. The reason for the relatively high expression levels of *Il2* and *Tgfb1* in the transported cell group needs to be investigated. Pro-inflammatory and fibrosis-related factors were expressed at early time points and then their expression decreased. There was no significant difference between the fresh and transported cell groups.

The kidney regeneration potency of the transported cells was evaluated via renal differentiation-related gene expression and functional assays. These expression levels of these genes gradually increased over time. Both cell-seeded groups showed similar expression values for *Wnt4*, *Pax2*, *Wt1*, *Emx2*, and *vWf*. Unexpectedly, the transported cell group showed significantly enhanced expressions of *Wnt4*, *Pax2*, *Wt1*, and *Emx2* at early time points. The expression patterns of these genes will be studied further in subsequent experiments. To evaluate renal function, serum BUN and creatinine concentrations [19] were measured. Both the fresh and transported cell groups showed similar BUN and creatinine concentrations, and the values were close to the normal range. Compared with the PLGA group, the values for both cell groups were significantly lower. Thus, the functional analysis confirmed that the

Fig. 4 Comparison of renal differentiation-related gene expression and functional recovery between freshly harvested cells and transported cells. **A** Renal differentiation-related gene expression. **B** Serum blood urea nitrogen (BUN) and creatinine levels. Ctrl, sham operated normal kidney; PL, kidney implanted with

poly(lactide-co-glycolide) (PLGA) scaffold (without cells); F, kidney implanted with freshly harvested cells seeded on a PLGA scaffold; T, kidney implanted with transported cells (under optimized conditions) seeded on a PLGA scaffold. * $P < 0.05$ and ** $P < 0.01$; $n = 5$ for each group

transported cells had equivalent regenerative potency to the freshly harvested cells.

Cells transported under conditions established in an *in vitro* experiment [3] showed recovery of total kidney volume, regeneration of glomerular/renal tubules, and low CD4/CD8 positive T cell infiltration. In addition, migration to distant organs and tumorigenicity were not observed over 40 weeks. In terms of functional recovery, the mean BUN and serum creatinine levels of the transported cell group were significantly decreased compared with those in the scaffold without cells group and were comparable to those of the freshly harvested cell group. Therefore, cells transported under optimized conditions can be used for cell

therapy similarly to freshly harvested cells, without deterioration of stem cell characteristics or tissue regeneration potency.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical statement This study was approved by the Ethics Committee of Kyungpook National University School of Medicine and all *in vivo* experimental protocols were approved by the Yeungnam University Institutional Animal Care and Use Committee (YUMC-AEC2015-003).

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