

Intraperitoneal Injection of Graphene Oxide Nanoparticle Accelerates Stem Cell Therapy Effects on Acute Kidney Injury

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Purpose: Graphene-based nanostructures have shown some degree of stem cell protection against cell death. Acute kidney injury (AKI) is a major cause of mortality in hospitalized patients. Here, graphene oxide (GO) was used to improve the efficacy of bone marrow-derived mesenchymal stem cells (MSCs) in the treatment of AKI induced by cisplatin, a chemotherapy medication used to treat a number of cancers.

Materials and Methods: Cisplatin-induced AKI was modeled in male rats. Intraperitoneal injection of MSCs mixed with GO, synthesized by graphite powder, H₂SO₄, and KMnO₄ was administered in modeled animals. Biochemical analysis of serum and histological and immunohistochemical (IHC) staining of kidney tissue samples were determined.

Results: Administration of GO nanoparticles suspended in MSCs reduced serum levels of creatinine (Cr) and blood urea nitrogen (BUN) in cisplatin-induced AKI in the experimental group compared to the control group. Histopathological evaluation also showed an improvement of morphological alterations of kidney, such as cellular proliferation, apoptosis and necrosis, cyst formation and intratubular debris in the experimental group compared to the control group. Our data revealed that GO injection alone without MSCs accelerated the improvement of the kidney injury induced by cisplatin.

Conclusion: This study demonstrated that suspended GO could enhance the efficacy of stem cells in the treatment of AKI. GO alone without stem cell accelerates the improvement of cisplatin-induced AKI.

Keywords: graphene oxide, kidney injury, stem cell

Introduction

Due to the unique role of the kidney, it is exposed to toxic compounds and therefore a frequent target of injury due to toxicity.¹ This can lead to Acute renal failure (AKI) that affects up to 7% of hospitalized patients. AKI is potentially reversible; it can be a determining factor in multiple organ failure as well. The mortality rate in hospital-acquired AKI ranges from 30% to 80%.² Common renal-protective approaches have presented partial therapeutic effects.³

Previous research have shown that the injection of MSCs can cooperate with kidney repair mainly by secreting paracrine factors.⁴ Transplantation of human bone marrow MSCs improves disease symptoms, decreases nephrotoxicity, and increases the survival of mice with AKI.⁵

The reason for the limited regenerative effects of MSCs is the loss of their adhesion after transplantation which induces MSC apoptosis and reduces the

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therapeutic effects of MSC transplantation.⁶ Sufficient number of stem cells and a tissue damage microenvironment are required by MSCs to promote tissue repair, thereby limiting their therapeutic efficacy in organ repair. MSCs secrete cytokines and growth factors with anti-inflammatory, immunosuppressive, antiapoptotic, and proliferative roles.⁷ This hypothesis demonstrates that the conditioned medium, where MSC-secreted growth factors and cytokines are present, could ameliorate renal tubular injury.⁷

Previously, we showed the effect of different times of intraperitoneal injection of human bone marrow MSC-conditioned medium on gentamicin-induced AKI and concluded that secretory factors of human MSCs can be partly protective against gentamicin-induced nephrotoxicity.⁸ In the present study, we sought a method to increase the effect of the stem cell-conditioned medium on AKI. Nano GO is a graphene derivative and a new class of carbon-based materials in a two-dimensional honeycomb structure. The hydrophilic nature of GO is the result of many hydroxyl groups on its surface which makes it resistant to electron transfer. It was proposed for biomedical applications due to its intrinsic optical properties, small size, easy use, and large specific surface area.^{7,10} These applications include biosensors,^{8,11} drug/gene delivery,^{9,12} and antibacterial effects.^{10,13} Moreover, GO reveals thermal, electrical, mechanical, and optical properties.^{11,12,14,15} Biomaterials are used to promote cell differentiation, attachment, and proliferation.¹⁶ Positive effects of GO on stem cell proliferation, adhesion and differentiation have been already confirmed.^{13,16} Graphene has attracted attention as a substrate for stem cell culture and is intended to stimulate the differentiation of multipotent adult stem cells. Recently, it was reported that graphene enhances the cardiomyogenic differentiation of human embryonic stem cells at least in part, due to its nano-roughness property.^{14,18} Previous studies have confirmed using graphene for neurogenesis and osteogenesis on 2D substrates.^{15,19} GO has been considered as a carrier for therapeutic proteins because of its more biocompatible and less toxicity effects.¹⁷ Previous researches have demonstrated that the implantation of GO particles did not exhibit obvious *in vivo* toxicity.^{20,21} It shows dose-dependent toxicity; its implantation in mice at a dosage less than 100 mg/kg body weight does not elicit obvious toxicity.²² Due to the presence of numerous oxygenated hydrophilic functional groups including phenol, carboxyl, etc. on its edges, graphene oxide yields a stable

suspension in water.²³ Oxygenated hydrophilic groups also provide GO with its unique physical, chemical, thermal, and electrical properties.^{24–26} These hydroxyl and carboxyl groups made it a negatively charged support.²⁷ The large number of hydroxyl groups on the surface of GO increases their biocompatibility, its large surface-to-volume ratio, and its specific topography, which leads to its high ability to absorb small molecules and extracellular matrix (ECM) proteins.^{28–30}

The aim of this work was to apply GO to increase the effects of the recovery of the conditioned medium in AKI. It seems likely that injecting MSC-conditioned medium containing growth factors and cytokines mixed with GO increases the recovery effects of injecting conditioned medium in AKI. We speculated that the adhesion of MSC-secreted growth factors to GO prior to injection enhances the range of improvements of AKI compared to those without GO groups.

We developed the fabrication of GO, and then used this suspended nanomaterial to improve the therapeutic efficacy of MSCs-conditioned medium injection on AKI. We developed a model of cisplatin-induced nephrotoxic injury in adult rats that presented with a typical AKI pattern, closely mimicking human AKI. The serum levels of BUN, Cr, cellular necrosis, formation of cysts, and intratubular debris in histological sections were analyzed in intraperitoneal injection of the MSC-conditioned medium, GO, and the MSC-conditioned medium mixed with suspended GO groups. Also, the expression of Ki-67 and terminal deoxynucleotidyl transferase dUTP

nick-end labeling (TUNEL) was analyzed in kidney cells of different groups. The Ki-67 nuclear protein is necessary for cellular proliferation³¹ and TUNEL staining is used in order to estimate of apoptotic cells.

Materials and Methods

Materials

Natural flake graphite was provided from “Qingdao Dingding Graphite Products.” GO was extracted from graphite using H₂SO₄ 98%, H₂O₂ 30%, and KMnO₄, all from Sigma-Aldrich. Octanol, styrene, benzoyl peroxide (BPO), and sodium dodecyl sulfate (SDS) were also obtained from Sigma-Aldrich.

Preparation of Water-Soluble GO

GO was derived from purified natural graphite according to the modified Hummer’s method.³² Graphite powder (0.5 g)

was added to 50 mL of H₂SO₄ 98% in ice bath, and KMnO₄ (2 g) was also added. The rate of addition was carefully controlled. The stirring was kept for 2 hrs at 10°C, followed by keeping for 1 hr at 35°C. It was then diluted with 50 mL of DI water in ice bath at 100°C. After 1 hr, it was further diluted to nearly 150 mL with DI water. 10 mL of H₂O₂ 30% was added to the mixture. The result was centrifuged and washed several times with HCl 5% aqueous solution. The resulting solid was dried at 60°C for 24 hrs.

Characterization

Samples were characterized by scanning electron microscopy (SEM, Philips XL30 microscope with an accelerating voltage of 25kV) and X-ray diffraction (XRD, Philips Xpert MPD, Co K irradiation). Furthermore, atomic force microscopy (AFM) as well as transmission electron microscopy (TEM) (PHILIPS, EM208S, Netherlands, at 100 kV of acceleration voltage) was used for morphology evaluation of Nano GO. Raman spectroscopy was applied to study layers and crystal structures of GO sheets. The Raman spectra of graphene films were obtained with a laser excitation of 632.8 nm at 1.7 mV. The few layers of GO were also characterized by AFM.

Design Model of Acute Kidney Injury

Male Wistar Albino rats weighing 180–220 g were housed under standard laboratory conditions (12 hrs of light/dark cycles) in a room with controlled temperature (24 ± 3°C), with the ethical principles of the National Institute of Health Guide for the Care and Use of Animals and the approval of the Ethics Committee at Kharazmi university (1912.544/2016). The study groups included the control group (n=6) not receiving any treatment; the cisplatin group (n=6) intraperitoneally receiving cisplatin at

a dosage of 5mg/kg body weight; the sham group (n=6) intraperitoneally receiving 500 µL saline on the 5th day after cisplatin injection; the MSC-conditioned medium group (n=6) receiving intraperitoneally condition medium after cisplatin injection; the GO group (n=6) receiving 1.5-mg/kg GO on the 5th day after intraperitoneal cisplatin injection; the MSC-conditioned medium + GO group (n=6) receiving 1.5 mg/kg GO + MSC-conditioned medium intraperitoneally on the 5th day after cisplatin injection. On the 9th day after cisplatin injection, kidney and blood samples of all rats were collected for biochemical and histological analysis.

Cell Culture

Human MSCs derived from bone marrow were obtained from the Royan institute, with the ethical principles of the National Institute of Health Guide for the Care and Use of Animals and the approval of the Ethics Committee at Royan institute (No EC.89.1061). The isolated MSCs (Figure 1) were transferred to DMEM (Dulbecco's Modified Eagle's Medium) containing 10% FBS and 1% penicillin-streptomycin. The cell supernatant was used in the treatment of animals after the incubation time. Each rat received MSC-conditioned medium intraperitoneally, which were injected at three equal volumes for three consecutive days.

Analyses of Apoptotic Cells

TUNEL staining was applied to observe apoptotic cells in the kidney tissue. The "In Situ Cell Death Detection Kit, POD" from Roche was used for this purpose. Kidney tissues were fixed with 10% (v/v) formaldehyde, embedded in paraffin, and sliced into 5µm sections. The paraffin-embedded kidney tissue was deparaffinized and

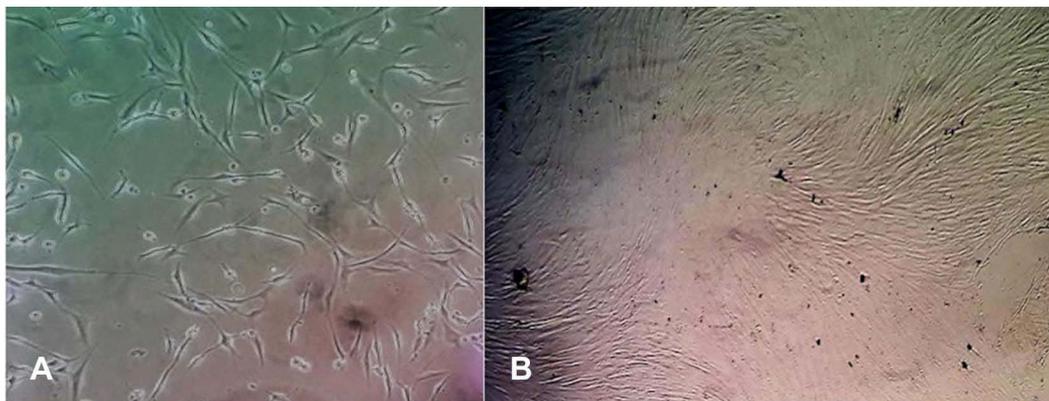


Figure 1 Phase contrast microscopy of bone marrow-derived MSCs at the first (A), and the third passage (B).

rehydrate through a graded series of ethanol and double-distilled water, washed in PBS, incubated for 20 mins at 37°C with proteinase K, permeabilized with 0.1% Triton x-100 and 0.1% sodium citrate, and finally incubated in TUNEL solution (450 μ L of label solution added with 40 μ L of enzyme solution) for 1 hr. After getting washed with PBS, the samples were observed under a fluorescent microscope.

Analyses of Ki-67 Immunostaining

Additional test to confirm cell proliferation such as Ki-67 was performed according to Buzatto et al.³²

Statistical Analysis

Statistical analysis was carried out using SPSS software ver. 16. Differences among groups were analyzed by one-way variance analysis (ANOVA) and the Dunnett's test.

P values less than 0.05 were considered as statistically significant.

Results

We have developed a novel and simple method to study the intraperitoneal injection of GO to improve the effects of MSC therapy on acute kidney injury in a rat model. Figure 2 shows the schematic representation of the GO synthesis and its characterization. Scanning electron microscopy (SEM) confirmed the synthesis of GO nanosheets with a quite smooth surface (Figure 2B), while atomic force microscopy (AFM) revealed isolated GO nanosheets well exfoliated and dispersed, with a thickness of about 1.5 nm each (Figure 2C). The results were further confirmed by TEM (Figure 2D). Based on the AFM and TEM results, the height of the prepared GO nanosheets was in the range of 1.5 to 2 nm, and their lateral size ranged from 100 nm to 2 μ m,

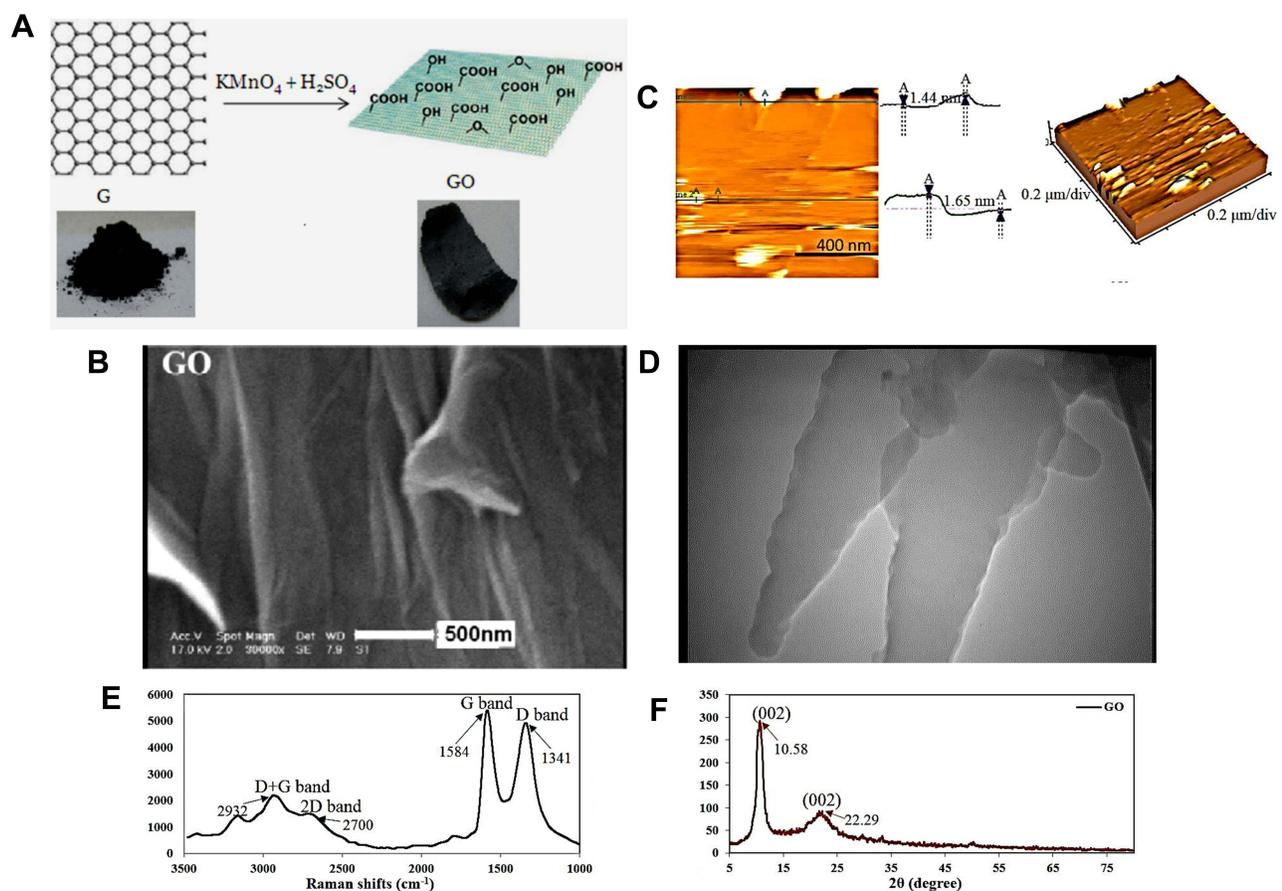


Figure 2 Synthesis of GO nanoparticles from graphite (A), SEM (B), AFM (C), TEM image of the synthesized GO nanosheets (D), Raman spectrum (E), and XRD pattern of GO nanosheets (F).

which is typical for GO nanosheets synthesized using Hummers method.³³

In addition, Raman spectroscopy showed distinctly disordered crystal structures and GO sheet layers with two characteristic GO peaks, namely, the D band around 1341 cm^{-1} and the G band around 1584 cm^{-1} (Figure 2E). The intensity ratio of D over G band (the R value = ID/IG) was calculated as 0.91, suggesting the presence of localized sp^3 defects.³⁴ Moreover, the x-ray power diffraction (XRD) pattern of the prepared GO showed a GO characteristic peak at $2\theta=10.58^\circ$ resulted from the diffraction on its 002 layer, along with a broad diffraction peak at 22.29° , which was interpreted in terms of short-range order in stacked graphene sheets (Figure 2F). Using the Debye–Scherrer equation (Eq. 1), the number of graphene layers in our sample was calculated as 8.³⁵

$$La = 0.89\lambda / (\beta_{002} \times \text{Cos}\theta_{002}),$$

$$L = La/d_{002},$$

La [stacking height], β [full-width half maxima-FWHM], n [number of graphene layers], d_{002} [interlayer spacing] were obtained by using the data from XRD patterns.

Although previous studies have shown that deionized water solutions of GO are highly stable, GO forms large clusters in the presence of salts. Such aggregation in mineral solutions is entirely dependent on GO concentration as well as the type and the amount of ions in the solution. In lower GO concentrations (below $6\mu\text{g/mL}$), the stable GO size has been measured.³⁶ In the present investigation, a nanoparticle dose of 1.5mg/kg was utilized, which was lower than the extreme of $6\mu\text{g/mL}$, and the possibility of GO nanosheet

aggregation was therefore very low. This hypothesis was confirmed using SEM analysis (Figure 2).

Our results showed significantly higher levels of serum Cr and BUN after cisplatin administration (Figure 3). Histological sections also showed that cisplatin enhanced necrosis, cyst formation and intratubular debris (Figures 4 and 5). Accordingly, a dose of 1.5 mg/kg GO was chosen for the treatment of AKI. However, a dose of 1.5 mg/kg GO did not alter Cr and BUN levels (Figures 6 and 7).

The effects of MSCs-conditioned medium, GO, and MSC-conditioned medium + GO in the treatment of AKI were compared. According to our findings, the treatment of animals in all these groups implied the improvement of AKI. MSC transplantation in passage 3 (Figure 1) had a regenerative effect on AKI (Figure 7). This was confirmed by blood serum biochemical tests and histological examinations of kidney tissue (Figure 4). MSCs-conditioned medium injection significantly repaired tissue damage; including cell necrosis, cyst formation, and intratubular debris. The results showed that AKI caused apoptosis and necrosis of epithelial cells in kidney tubules (Figures 4 and 5).

Biochemical and histological markers implied that the selected GO dose of 1.5 mg/kg made no significant changes in the biochemical markers of the blood and histological features (Figure 7). The MSC-conditioned medium + GO resulted in a higher level of improvement of AKI compared to the others (Figures 4 and 7). In addition, the GO-induced improvement was greater than that of the MSC-conditioned medium group (Figures 4 and 7). The serum levels of BUN and Cr in the conditioned medium + GO group were lower than those of the MSC group. Histological findings showed that cellular necrosis, formation of cysts, and intratubular debris were

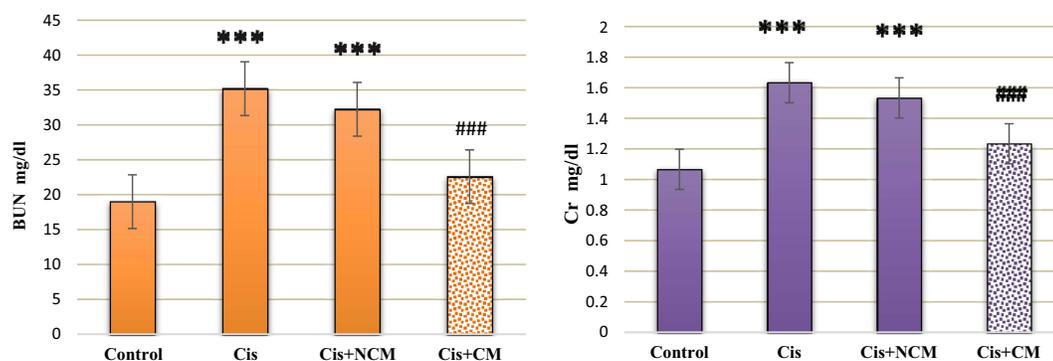


Figure 3 Intraperitoneal injection of conditioned medium (CM) significantly reduced the level of serum Cr (mg/dL) in AKI rats (A). Values with asterisks (***) were significantly different from the control group (***P<0.001). Values with squares (####) were significantly different from the cisplatin group (####P<0.001). Intraperitoneal injection of CM on the ninth day after cisplatin injection significantly reduced the level of serum BUN (mg/dL) in AKI rats (B). Values with asterisks (***) were significantly different from the control group (***P<0.001). Values with squares (####) were significantly different from the cisplatin group (####P<0.001, n=6).

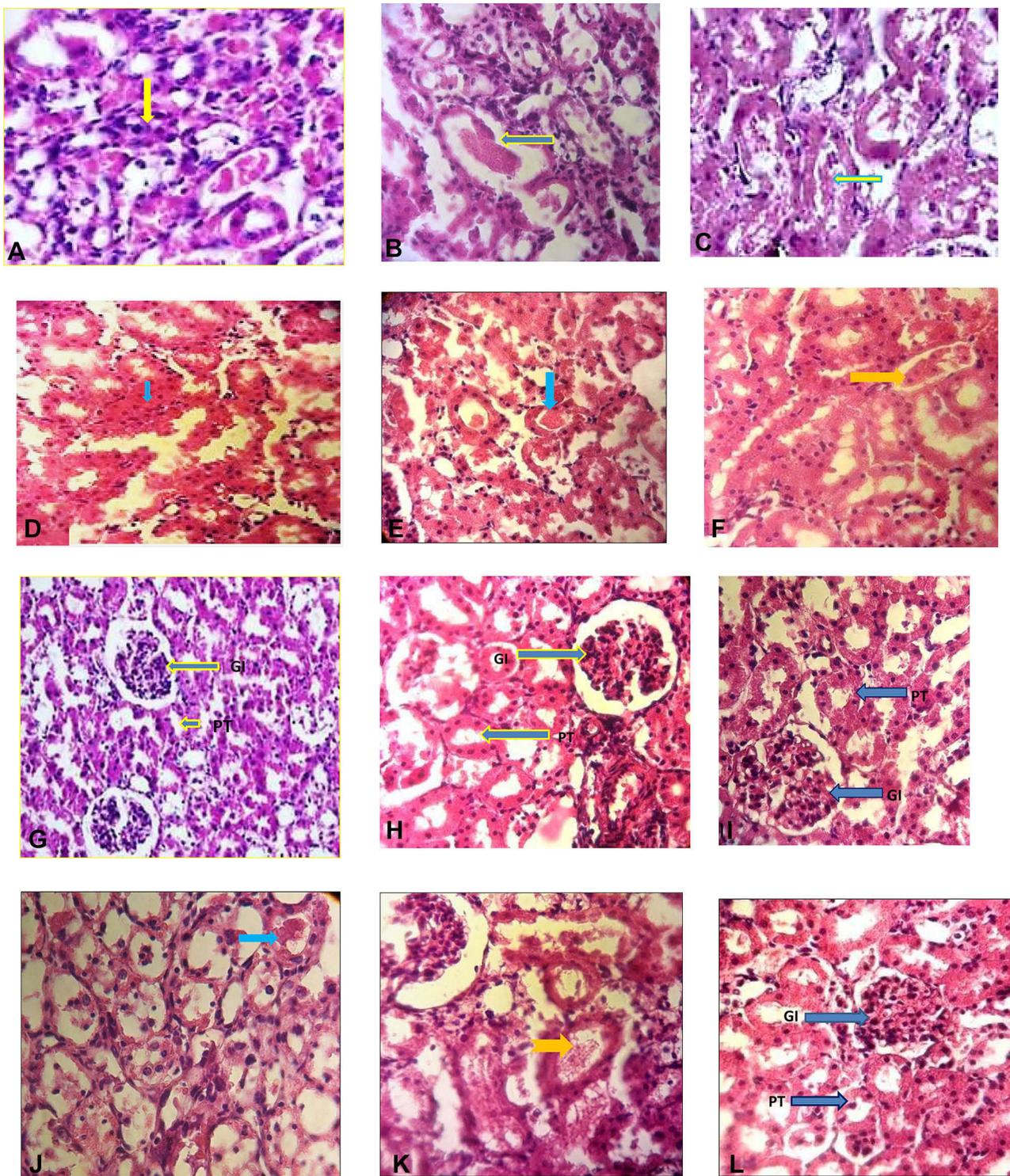


Figure 4 The study of histopathologic analysis of the kidney of various tested rats after cisplatin induction. Histological section of cisplatin group; **A** (necrosis), **B** (cast hyaline), and **C** (intratubular debris). Histological sections of the kidneys of the control groups are normal and non-harmful (**G**). In the kidneys of the first experimental group, receiving DMEM + FBS medium (**D**, **E**, **F**), necrosis (green arrow), cast hyaline (blue arrow), and intra-tubular debris (yellow arrow) were found. In the kidneys of the second experimental group (**H**), receiving 5×10^6 stem cells conditioned medium, it was found only Intra-tubular debris. In the kidneys of the third experimental group (**I**), receiving 5×10^6 stem cells + GO, none of the damage was observed. In the kidneys of the fourth experimental group (**J**, **K**), receiving GO, there was found cast hyaline (**J**), and Intra-tubular debris (**K**). In the kidneys of receiving GO (without cisplatin injection), none of the damage was observed (**L**) and GO had no toxic effect. (**H**, **E**) staining $\times 400$.

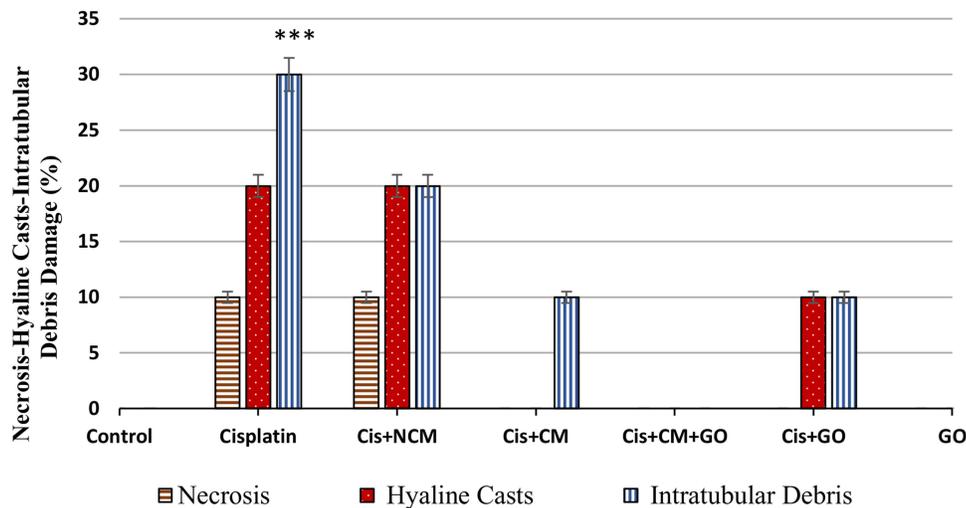


Figure 5 Histopathologic analysis of the kidney of AKI induced by cisplatin of tested rats. Intratubular debris increased in cisplatin group compared to the other groups (**P<0.001).

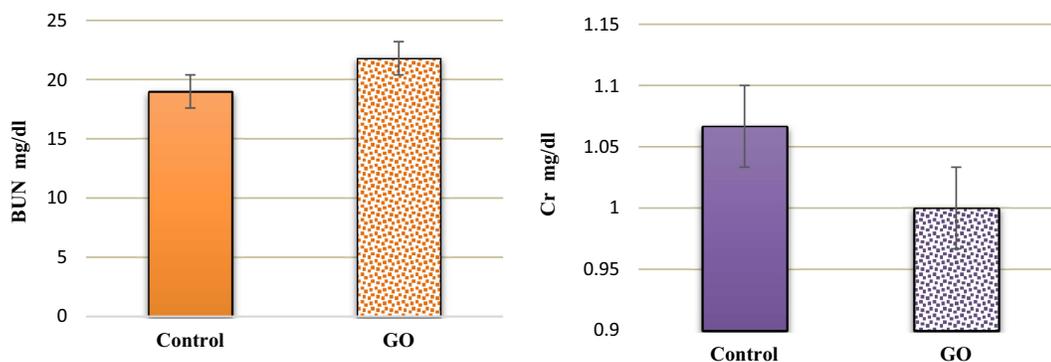


Figure 6 Intraperitoneal injection of GO insignificantly reduced the level of serum Cr and BUN on the ninth day after cisplatin injection compared to the control group (n=6).

significantly lower in the MSC-conditioned medium + GO compared to those in the group receiving only MSCs (Figures 4 and 7). They also revealed a reduction in necrosis and cyst formation in the MSCs-conditioned medium + GO group compared to the MSC group (Figure 5). We observed that the effects of GO alone on the treatment of AKI are less than those of the MSCs-conditioned medium. Biochemical and histological results confirmed these findings (Figures 4 and 7). Our SEM images showed that the edges of GO hybrid exhibit good connections with MSCs and biomolecules such as growth factors (Figure 8). Comparison of MSCs cultured with and without GO showed MSCs attached to GO edges, forming a single unit (Figure 8).

In the present study, we compared the Ki-67 expression in the kidney tissue cells of the control and the AKI rat model receiving GO and the GO + stem cell-conditioned medium. Figure 9 indicates an increase in

the number of Ki-67 positive cells in the kidney of the AKI rat model treated with GO or the GO + MSC-conditioned medium compared to the AKI rat model receiving no treatment. Ki-67 positive cells in the AKI rat model treated with MSCs + GO group (40%) were more than those of the GO (30%) and cisplatin (10%) groups. TUNEL staining confirmed a widespread tubular cell death due to apoptosis following the cisplatin-induced injury. Our results indicated a decrease in the number of apoptosis cells in the groups treated with GO (30%) and MSCs + GO (10%) following cisplatin injection compared to the cisplatin group (50%) (Figure 10).

Discussion

The results of the present study indicated that the MSC-derived conditioned medium reduced the level of serum Cr and BUN (mg/dL) in a rat model of cisplatin nephrotoxicity. Such biomedical results were also confirmed through

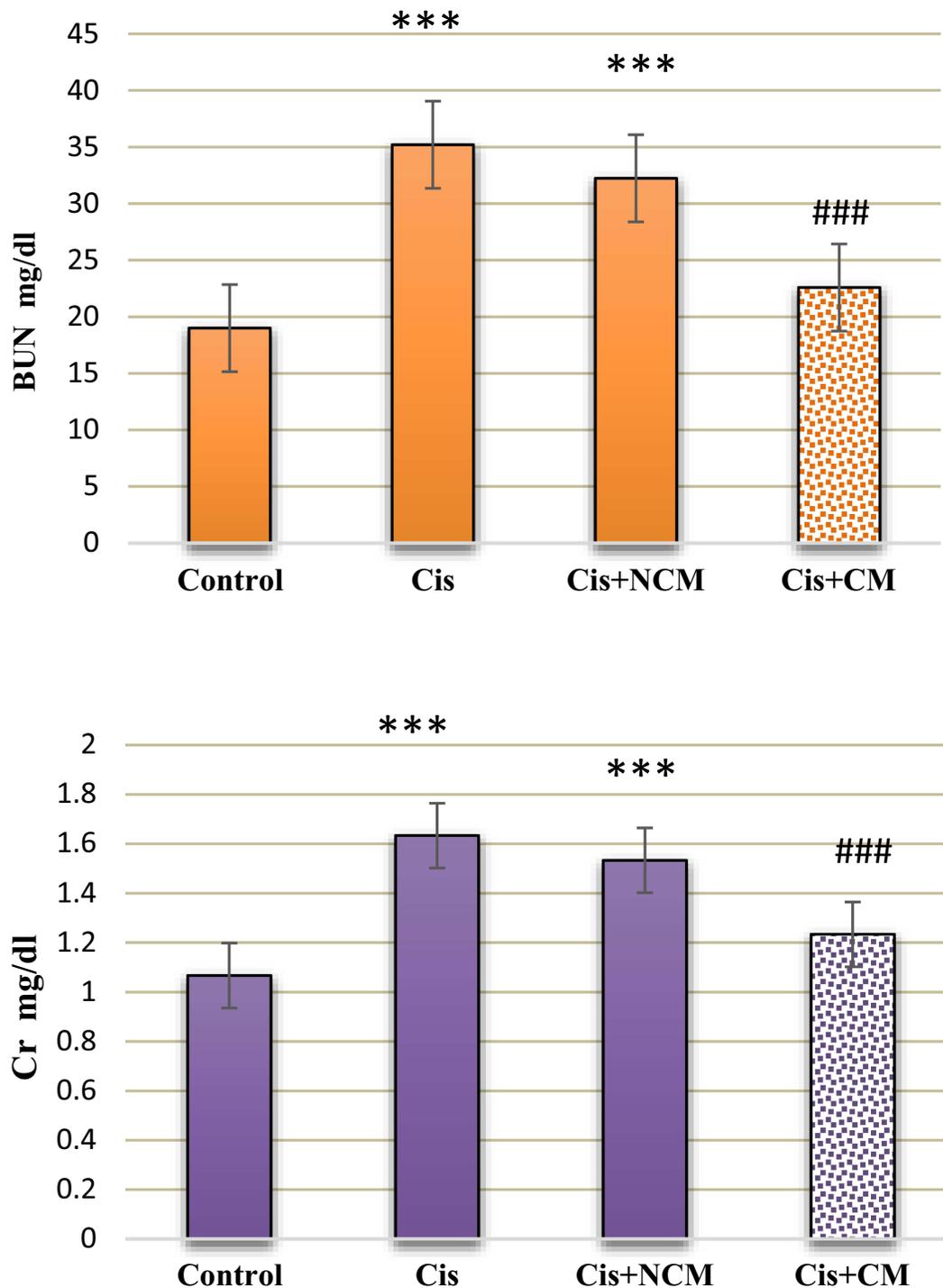


Figure 7 Intraperitoneal injections of the conditioned medium (CM) + GO on the ninth day after cisplatin injection significantly reduced the level of serum Cr and BUN (mg/dL) in AKI rats. Values with asterisks (***) were significantly different from the control group (***) $P < 0.001$). Values with squares (###) were significantly different from the cisplatin group (###) $P < 0.001$, $n = 6$.

histological analysis. We showed that the GO + MSC-derived conditioned medium would prevent kidney cells from undergoing apoptosis, thereby protecting them against tubular injury. The MSC-derived conditioned medium containing growth factors and cytokines promotes

antiapoptotic and proliferative effects.¹ Secretory factors derived from MSCs can be partly protective against cisplatin-induced nephrotoxicity.⁸ The results of the present study showed a method to increase the effect of the stem cells on the treatment of the AKI animal model. Our

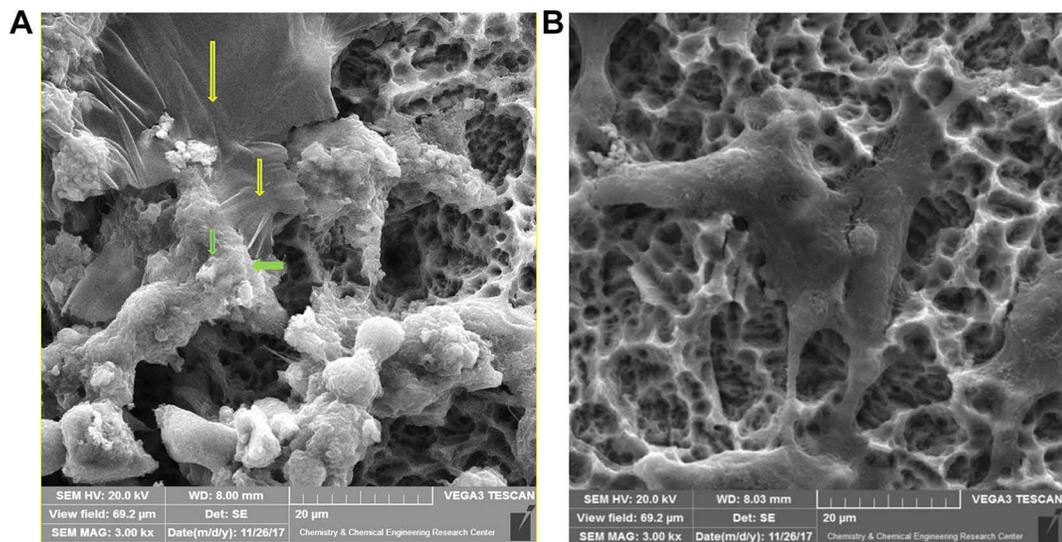


Figure 8 SEM images of interactions among GO edges, MSCs, and growth factors derived from stem cells (A). Arrows indicate the attachment of stem cells and growth factors secreted from MSCs (green) to the edges of the silica-GO layer (yellow). In the absence of GO, the aggregation of MSCs was not sufficiently noticeable (B).

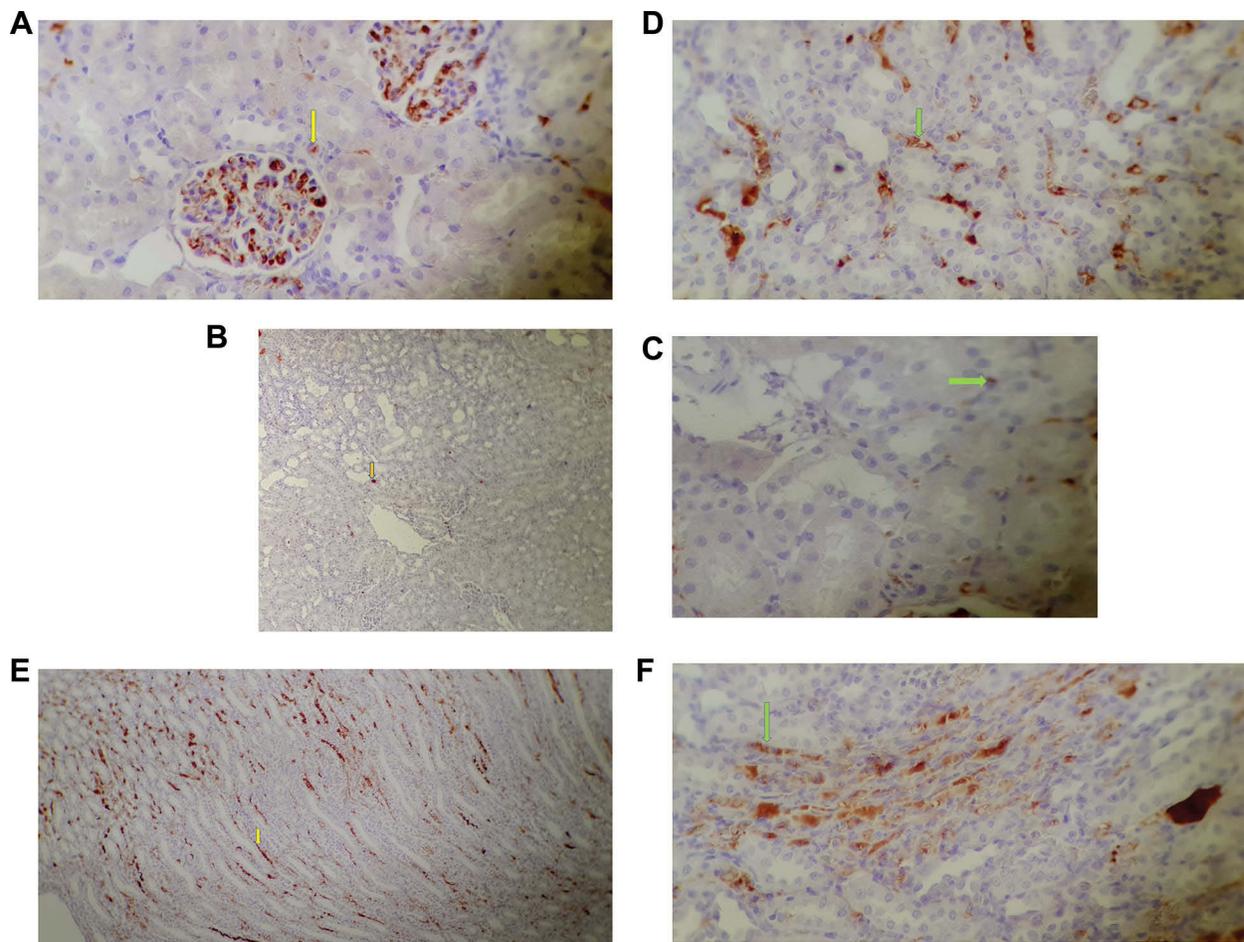


Figure 9 Ki-67 staining in the kidney of various tested rats. (A) Control groups (not receiving any treatment; magnification 400×), (B, C) The group receiving cisplatin; magnification 100 and 400×, (D) The group receiving 1.5 mg/kg GO after cisplatin injection; magnification 200×, (E, F) The group receiving 1.5 mg/kg GO + MSC-conditioned medium after cisplatin injection; magnification 100 and 200×. Arrow indicates positive nuclear staining for Ki-67.

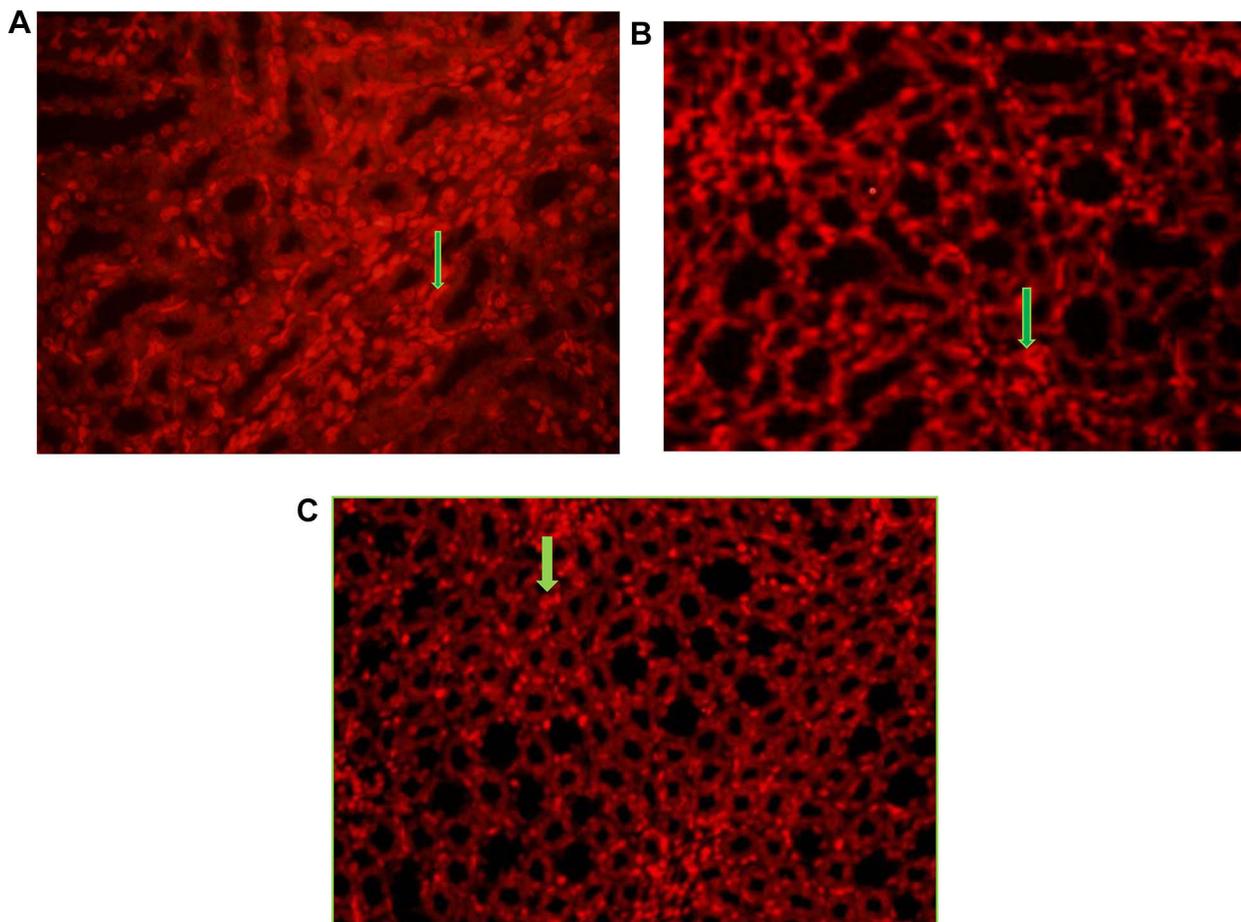


Figure 10 (A) TUNEL staining of the kidney of various tested rats after cisplatin induction. (B, C) Immuno histological sections of the kidneys of the GO and MSC-conditioned medium + GO groups indicated a decrease of apoptotic cells (arrow) compared to the cisplatin group ($\times 400$).

findings indicated that both GO and the MSC-conditioned medium + GO injections improved the effects of MSCs. Today, some biomaterials are used to promote cell differentiation, attachment, and proliferation.^{16,37} Previous studies have proved that the use of graphene-based materials facilitates and accelerates stem cell differentiation.^{38,39} Positive effects of GO on the proliferation, adhesion and differentiation of stem cells have already been confirmed.^{13,16} Our biomedical and histological results showed the positive effects of GO and the MSC-conditioned medium + GO on the treatment of AKI. GO and the MSC-conditioned medium + GO could improve the injured kidney model through survival of kidney cells as the result of their limited apoptosis and enhanced proliferation. We found that significantly more kidney cells were stained with KI-67 in the GO + MSC-conditioned medium and GO groups following cisplatin induction. The presence and activity of KI-67 are greatly associated with cell proliferation.³¹ The abundance of KI-67 positive cells

might strongly indicate the positive effects of GO on tissue cells' proliferation for kidney repair. Also, it could be concluded that GO can accelerate the effects of MSC therapy in the treatment of AKI.

The most important factor associated with some pathologies in AKI is oxidative stress.³⁸ Oxidative stress-related AKI is associated with a decrease in endogenous antioxidant levels, and an increase in levels of reactive oxygen species (ROS) as a toxic factor and reactive nitrogen species.⁴⁰ At higher doses, ROS act as reactive molecules that can damage growth factors and disrupt cellular integrity.⁴¹ ROS not only prevent cell adhesion, but also induce cell apoptosis.⁴¹ The use of MSCs to treat damaged tissue is normally hindered by generation of ROS.⁴² Some indicators of cell function, such as viability, protein secretion, and cell adhesion were all enhanced in MSCs mixed with GO in the presence of ROS, indicating that GO has a capability to protect MSCs from ROS, even in harsh conditions.⁴¹ As

expected, when the GO + MSC-conditioned medium was delivered to the damaged kidney, cells showed a decreased kidney tissue apoptosis. We examined the TUNEL expression of kidney cells in different groups to analyze their apoptotic activity. TUNEL staining confirmed a widespread tubular cell death due to apoptosis following injury induced by cisplatin. Our results indicated a decrease in apoptosis cells in the animal model of AKI treated with Ki GO and MSCs + GO. Positive nuclear staining for Ki-67 marker occurred

in greater than 40% of the kidney cells of AKI models treated with GO + MSC-conditioned medium, which indicated a high proliferative index compare to the GO (32%) and control (20%) groups, respectively. The results of IHC staining showed that GO plays a proliferative and antiapoptotic roles in the repair of damaged tissue.

The GO's specific topography leads to its high ability to absorb small molecules and ECM proteins.²⁸ These properties make GO very attractive for various applications including drug delivery.^{29,30} The hydrophilic nature of GO is the result of many hydroxyl groups on its surface which makes it resistant to electron transfer.⁴² Our SEM images showed that the edges of GO exhibit good connections with MSCs and biomolecules such as growth factors (Figure 8). Comparison of MSCs cultured with and without GO showed that MSCs attached to GO edges, forming a single unit. It appears that GO as a carrier with low toxicity effects plays an important role in the delivery of growth factors to the damaged site, and thereby in the improvement of the therapeutic efficacy of MSCs.

Conclusion

The present study indicated that treatment with MSCs, GO, and GO + MSCs helped to improve AKI. GO surface improved adsorption of MSCs, secreted growth factors from MSCs, and important factors in the blood. It enabled MSCs to better reach and interact with damaged and healthy kidney stem cells. Moreover, GO enhanced cells' interactions with each other as well as with ECM. In other words, GO improved recovery through stem cell transplantation in the kidney. It appeared that GO increases the effects of stem cell therapy in AKI, and that GO nanohybrid could be used in combination with stem cells for the treatment of diseases, both in vivo and in vitro. We concluded that GO accelerates absorption and loading of growth

factors, and therefore improves the regenerative and protective effects of stem cells.

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Disclosure

The authors report no conflicts of interest in this work.

References

- Moghadasi R, Mutsaers HA, Azarnia M, et al. Mesenchymal stem cell-conditioned medium accelerates generation of human renal proximal tubule epithelial cells after gentamicin toxicity. *Exp Toxicol Pathol*. 2013;65(5):595–600. doi:10.1016/j.etp.2012.06.002
- Schrier RW, Wang W, Poole B, et al. Acute renal failure: definitions, diagnosis, pathogenesis, and therapy. *J Clin Invest*. 2004;114:5–14. doi:10.1172/JCI200422353
- Pabla N, Dong Z. Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. *Kidney Int*. 2008;73:994–1007. doi:10.1038/sj.ki.5002786
- Bi B, Schmitt R, Israilova M, et al. Stromal cells protect against acute tubular injury via an endocrine effect. *J Am Soc Nephrol*. 2007;18:2486–2496. doi:10.1681/ASN.2007020140
- Morigi M, Inrona M, Imberti B, et al. Human bone marrow mesenchymal stem cells accelerate recovery of acute renal injury and prolong survival in mice. *Stem Cells*. 2008;26:2075–2082. doi:10.1634/stemcells.2007-0795
- Park J, Kim B, Han J, et al. Graphene oxide flakes as a cellular adhesive: prevention of reactive oxygen species mediated death of implanted cells for cardiac repair. *ACS Nano*. 2015;9:4987–4999. doi:10.1021/nn507149w
- Humphreys BD, Bonventre JV. Mesenchymal stem cells in acute kidney injury. *Ann Rev Med*. 2008;59:311–325. doi:10.1146/annurev.med.59.061506.154239
- Abedi A, Azarnia M, Jamali M, et al. Effect of different times of intraperitoneal injections of human bone marrow mesenchymal stem cell conditioned medium on gentamicin-induced acute kidney injury. *Urol J*. 2016;13:2708.
- Gonçalves G, Vila M, Portolés MT, et al. Nano-graphene oxide: a potential multifunctional platform for cancer therapy. *Adv Healthcare Mater*. 2013;2:1072–1090. doi:10.1002/adhm.201300023
- He S, Song B, Li D, et al. A graphene nanoprobe for rapid, sensitive, and multicolor fluorescent DNA analysis. *Adv Func Mater*. 2010;20:453–459. doi:10.1002/adfm.200901639
- Kim H, Namgung R, Singha K, et al. Graphene oxide–polyethylenimine nanoconstruct as a gene delivery vector and bioimaging tool. *Bioconj Chem*. 2011;22:2558. doi:10.1021/bc200397j
- Akhavan O, Ghaderi E. Toxicity of graphene and graphene oxide nanowalls against bacteria. *ACS Nano*. 2010;4:5731–5736. doi:10.1021/nn101390x
- Balandin A, Ghosh S, Bao WZ, et al. Superior thermal conductivity of single-layer grapheme. *Nano Lett*. 2008;8:902–907. doi:10.1021/nl0731872

14. Lee C, Wei XD, Kysar JW, et al. Measurement of the elastic properties and intrinsic strength of monolayer grapheme. *Science*. 2008;321:385–388. doi:10.1126/science.1157996
15. Park SY, Park J, Sim SH, et al. Enhanced differentiation of human neural stem cells into neurons on grapheme. *Adv Mater*. 2011;23:263–267. doi:10.1002/adma.201101503
16. Wagner AS, Glenske K, Henss A, et al. Cell behavior of human mesenchymal stromal cells in response to silica/collagen based xerogels and calcium deficient culture conditions. *Biomed Mater*. 2017;4(12):045003. doi:10.1088/1748-605X/aa6e29
17. Lee TJ, Park S, Bhang SH, et al. Graphene enhances the cardiomyogenic differentiation of human embryonic stem cells. *Biochem Biophys Res Comm*. 2014;12:174–180. doi:10.1016/j.bbrc.2014.08.062
18. Kim TH, Lee T, El-Said WA, et al. Graphene-based materials for stem cell. *Appl Mater*. 2015;8:8674–8690.
19. Park J, Kim IY, Patel M, et al. 2D and 3D hybrid systems for enhancement of chondrogenic differentiation of tonsil-derived mesenchymal stem cells. *Adv Funct Mater*. 2015;25:2573–2582. doi:10.1002/adfm.201500299
20. Kanakia S, Toussaint JD, Mullick, et al. Dose ranging, expanded acute toxicity and safety pharmacology studies for intravenously administered functionalized graphene nanoparticle formulations. *Biomaterials*. 2014;35:7022–7031. doi:10.1016/j.biomaterials.2014.04.066
21. Yang K, Gong H, Shi XZ, et al. In vivo biodistribution and toxicology of functionalized nano-graphene oxide in mice after oral and intraperitoneal administration. *Biomaterials*. 2013;34:2787–2795. doi:10.1016/j.biomaterials.2013.01.001
22. Wang K, Ruan J, Song H, et al. Biocompatibility of graphene oxide. *Nanoscale Res Lett*. 2011;6:8.
23. Kumeria T, Bariana M, Alalhi T, et al. Graphene oxide decorated diatom silica particles as new nano-hybrids: towards smart natural drug microcarriers. *J Mater Chem B*. 2013;1:6320. doi:10.1039/c3tb21051k
24. Dreyer DR, Park S, Bielawski CW, et al. The chemistry of graphene oxide. *Chem Soc Rev*. 2010;39:228. doi:10.1039/B917103G
25. Chung C, YK K, Shin D, et al. Biomedical applications of graphene and graphene oxide. *Acc Chem Res*. 2013;46(10):2211–2224. doi:10.1021/ar300159f
26. Wang Y, Li Z, Wang J, et al. Graphene and graphene oxide: biofunctionalization and applications in biotechnology. *Trends Biotechnol*. 2011;29:205–212. doi:10.1016/j.tibtech.2011.01.008
27. Yua L, Maa F, Dinga X, et al. Silica/graphene oxide nanocomposites: potential adsorbents for solid phase extraction of trace aflatoxins in cereal crops coupled with high performance liquid chromatography. *Food Chem*. 2018;245:1018–1024. doi:10.1016/j.foodchem.2017.11.070
28. Kim J, Choi KS, Kim Y, et al. Bioactive effects of graphene oxide cell culture substratum on structure and function of human adipose derived stem cells. *J Biomed Mater Res. Part A*. 2013;101(12):3520–3530. doi:10.1002/jbm.a.34659
29. Sun X, Liu Z, Welsher K, et al. Nano-graphene oxide for cellular imaging and drug delivery. *Nano Res*. 2008;1:203. doi:10.1007/s12274-008-8021-8
30. Bai H, Li C, Wang X, et al. A pH-sensitive graphene oxide composite hydrogel. *Chem Commun*. 2010;46:2376. doi:10.1039/c000051e
31. Madani SH, Ameli S, Khazaei S, et al. Frequency of Ki-67 (MIB-1) and P53 expressions among patients with prostate cancer. *Indian J Pathol Microbiol*. 2011;54:688–691. doi:10.4103/0377-4929.91492
32. Buzatto AB, Elias F, Franzoni MS, et al. Long-term survival of a cat with primary leiomyosarcoma of the urinary bladder. *Vet Sci*. 2019;6:60. doi:10.3390/vetsci6030060
33. Hummers WS Jr, Offeman RE. Preparation of graphitic oxide. *J Am Chem Soc*. 1958;80(6):1339. doi:10.1021/ja01539a017
34. Stöber W, Fink A, Bohn E. Controlled growth of monodisperse silica spheres in the micron size range. *J Colloid Interface Sci*. 1968;26(1):62–69. doi:10.1016/0021-9797(68)90272-5
35. Saner B, Dinç F, Yürüm Y. Utilization of multiple graphene nano sheets in fuel cells: the effect of oxidation process on the characteristics of graphene nanosheets. *Fuel*. 2011;90(8):2609–2616. doi:10.1016/j.fuel.2011.03.040
36. Palmieri V, Bugli F, Carmela LM, et al. Bacteria meet graphene: modulation of graphene oxide nanosheet interaction with human pathogens for effective antimicrobial therapy. *ACS Biomater Sci Eng*. 2017;3(4):619–627. doi:10.1021/acsbomaterials.6b00812
37. Foroutan T, Mousavi M. The effects of zinc oxide nanoparticles on differentiation of human mesenchymal stem cells to osteoblast. *Nanomed J*. 2014;1(5):308–314.
38. Hong SW, Lee JH, Kang SH, et al. Enhanced neural cell adhesion and neurite outgrowth on graphene-based biomimetic substrates. *BioMed Res Int*. 2014;2014:212149.
39. Foroutan T, Nazemi N, Tavana M, et al. Suspended graphene oxide nanoparticle for accelerated multilayer osteoblast attachment. *J Biomed Mater Res Part A*. 2018;106:293–303. doi:10.1002/jbm.a.36231
40. Palipoch S. Review of oxidative stress in acute kidney injury: protective role of medicinal plants-derived antioxidants. *AJTAM*. 2013;10(4):88–93. doi:10.4314/ajtam.v10i4.15
41. Mirosou M, Jayawardena TM, Schmeckpeper J, et al. Paracrine mechanisms of stem cell reparative and regenerative actions in the heart. *J Mol Cell Cardiol*. 2011;50:280–289. doi:10.1016/j.yjmcc.2010.08.005
42. Kim TH, Lee T, El-Said WA, et al. Graphene-based materials for stem cell applications. *Materials*. 2015;8:8674–8690. doi:10.3390/ma8125481

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