Supporting Information

Construction of a 3D rGO-Collagen Hybrid Scaffold for Enhancement of the Neural Differentiation of Mesenchymal Stem Cells

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This PDF file contents including as follows:

S1. Preparation and characterization of GO

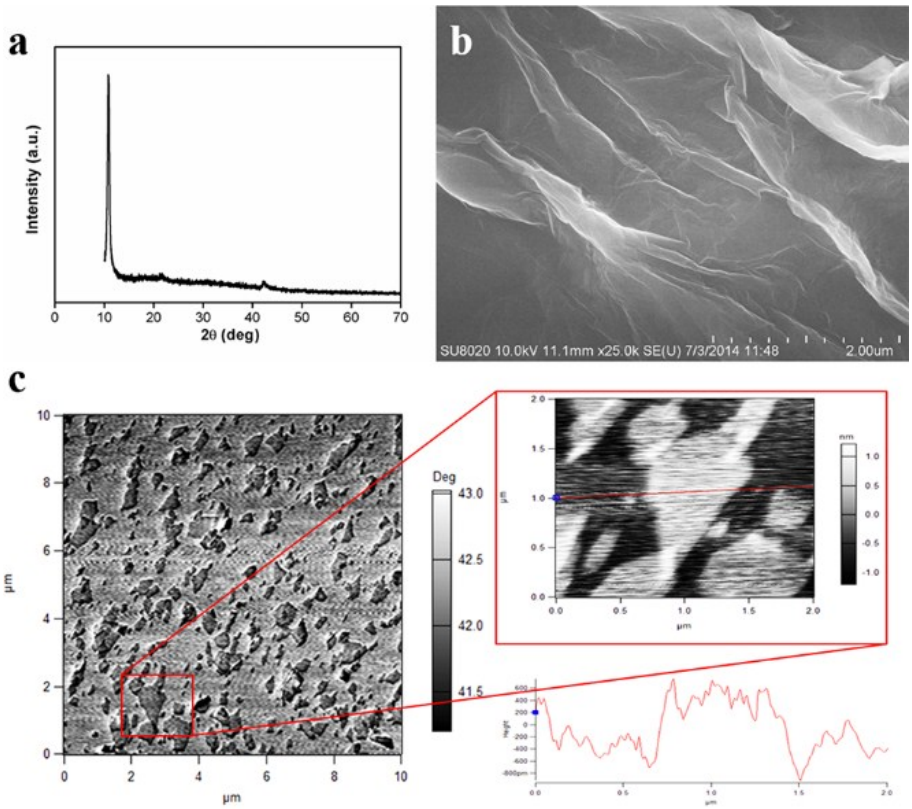

Figure S1. X-Ray Diffraction (XRD) pattern of GO power (a), SEM image of GO (b), Atomic Force Microscopy (AFM) of GO nanosheets (c).

The graphene oxide (GO) was prepared by the modified Hummers' method. Briefly, 12.5ml concentrated H_2SO_4 was heated to 80°C, 2.5g $K_2S_2O_8$ and 2.5g P_2O_5 were added to the acid. 3g graphite powder (Alfa Aesar, Tianjin) was added to the solution and kept at 80°C for 5h. The mixture was then cooled, diluted with deionized water, filtered using filter papers, further rinsed with additional 1000ml deionized water to remove residual reactants, and then dried in air.[1] The pre-treated graphite powder was transferred into a flask with 72ml concentrated H_2SO_4 and 1.5g $NaNO_3$, and chilled to 0°C using an ice bath. 9g $KMnO_4$ was slowly added into the mixture while magnetic stirring, keep the temperature below 20°C. The flask was then moved to a 35°C water bath and left for 30min. The paste was brownish grey in color. At the end of 30min, 138ml deionized water was slowly added to the flask. After dilution, transferred the flask into another 98°C water bath for 15min, the suspension was further diluted with 420ml deionized water and treated with 3% hydrogen peroxide to reduce the residual $KMnO_4$. The color of the solution turned bright yellow. This suspension was filtered and rinsed with 2L 5%HCl solution to remove residual salts. The yellowish-brown filter cake was dehydration at 40°C in vacuum. GO powder was dispersed in deionized water, the remaining salt impurities were removed by dialysis for 7days, exchanged the deionized water daily, finally get the GO solution with a certain concentration of 0.25mg/ml.[2]

In the typical characterizations of GO, the XRD pattern of GO (a), the characteristic diffraction peak of GO was observed at 10.8°. It showed the GO with a high purity, we can see a layer of GO with wrinkles in the SEM image (b). Then we measured the thickness of GO nanosheets using AFM, the GO was with an excellent dispersity in aqueous solution and the thickness of GO nanosheets was about 1.5nm. All the results demonstrated the success of preparing GO by the modified Hummers' method with excellent

properties.

S2. Schematic of prepare PADM-rGO hybrid scaffold

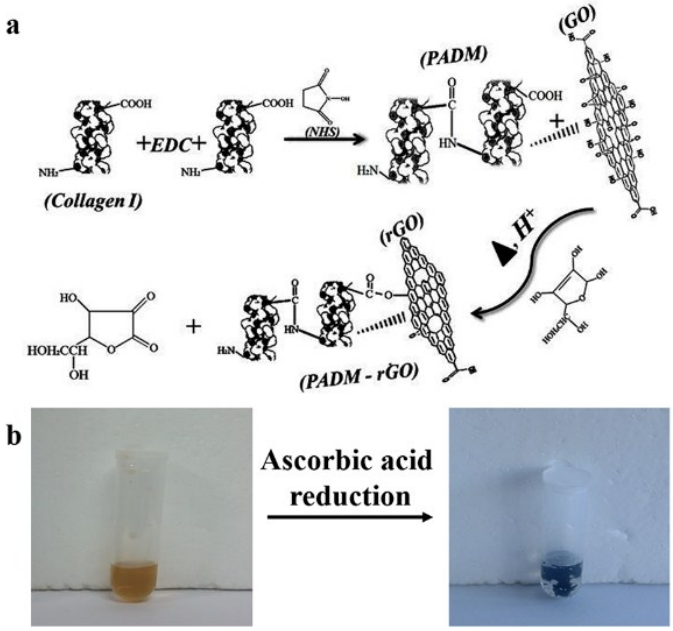

Figure S2. (a) Schematic of prepare PADM-rGO hybrid scaffold, (b) the state of GO after reduced by ascorbic acid.

The schematic of prepare PADM-rGO has been shown in Figure S2a. The EDC/NHS crosslinking process aims to reinforce the decellularized organ matrix, and EDC is a zero-length crosslinker which does not form harmful crosslink bonds. In crosslinking process, there should be amide bonds forming between amino and carboxyl groups. GO consists of graphene sheets which are chemically functionalized with hydroxyl and epoxy groups.[3] Carbonyl groups are also present as carboxylic acids along the sheet edges. The existence of oxygen-containing groups makes GO hydrophilic and dispersive into some polar solvents forming intercalated composites with polar molecules through the strong interaction. In addition, the oxygen-containing groups on GO are very easy to be assembled, enable great interactions with various proteins, organics through covalent, electrostatic, and hydrogen bonding.[4] A large gap zeta potential between GO and PADM make sure the GO nanosheets can easily adhere on the surface of PADM, ascorbic acid, as the reducing agent (show in a), not only as a green reducing agent but also provide an acid environment for the esterification reaction between collagen and GO. From the Figure S2b, the state of rGO was stable after the GO reduced by ascorbic acid, it also demonstrated that once the GO adhered on the surface of PADM, except the chemical bonding between the rGO and PADM, the adsorption capacity of rGO film on the PADM surface is another guarantee of rGO film stable on PADM.

S3. SEM images of ascorbic acid reduced GO, the channel surface of PADM-rGO hybrid scaffold, the Raman spectroscopy of highly reduced GO sheet-PADM.

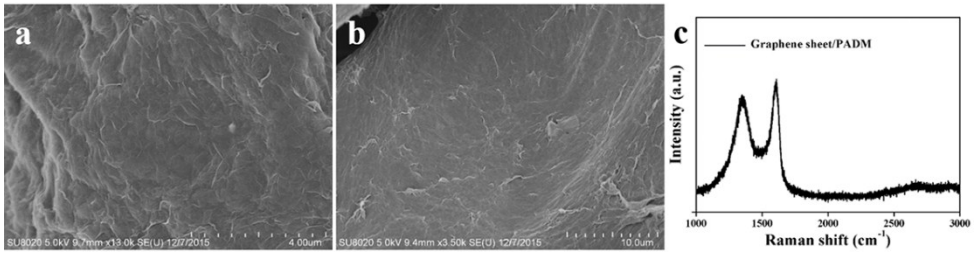

Figure S3. SEM images of ascorbic acid reduced GO (a), the channel surface of PADM-rGO hybrid

scaffold, (c) is the Raman spectroscopy of highly reduced GO sheet-PADM.

From the SEM images of SEM images of ascorbic acid reduced GO (a), the channel surface of PADM-rGO hybrid scaffold (b), they have similar surface topographies, it demonstrated that the rGO is well covered on the surface of the PADM. Highly reduced GO was obtained by $NaBH_4$ as the reduce agent, the highly reduced GO sheets can easily adhere on the surface of PADM. The Raman spectroscopy of highly reduced GO sheet-PADM (c) shows a more week of intensity of D band from the ascorbic acid reduced GO sheet-PADM.

S4. I-V test of PADM-rGO hybrid scaffold

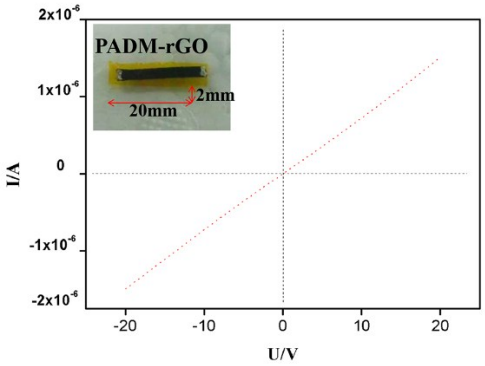

Figure S4. I-V test of PADM-rGO hybrid scaffold.

I-V test assay was performed by Yiye-EB-4 probe station. It showed that after assembling a layer of rGO on the PADM, the insulated collagen matrix become a conductive one.

S5. Zeta potential of GO solution and PADM suspension, FT-IR spectrum of PADM and PADM-rGO

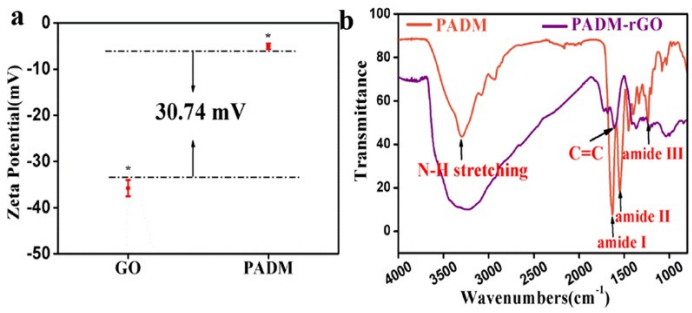

Figure S5. Zeta potential of GO solution and PADM suspension (a), FT-IR spectrum of PADM and PADM-rGO (b). (*p ≤ 0.05, **p ≤ 0.01, n=3)

GO has excellent water-solubility, the Zeta potential of 0.25 mg/ml GO solution was tested. For the PADM, the Zeta potential was tested after made 1 g PADM sample into 2 ml homogeneous suspension by crushing.

S6. Purity analysis of the isolated MSCs

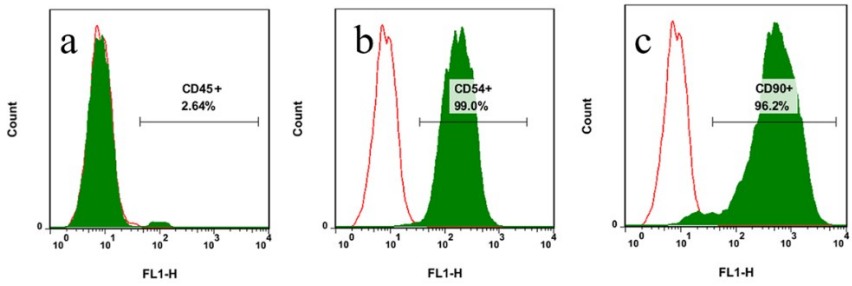

Figure S6. Flow cytometry purity analysis of the MSCs. Red peak, the control of mouse IgG. Green peak,

CD45 (a), CD54 (b), CD90 (c) staining.

The third passage cells (5×10^5) were incubated with 0.5 μg fluorescein isothiocyanate (FITC)-conjugated mouse anti-rat monoclonal antibodies specific to rat CD45 (BD), CD54 (BD), CD90 (BD), for 1 h at 4 °C.

MSCs express CD54 and CD90, but not CD45. The result showed that 97.36% of the cells were CD45 negative (a), 99.0% were CD54 positive (b) and 96.2% were CD90 positive (c), we can calculate that the purity of the MSCs was over 90%.

S7. Staining of actin filaments after the cells seeded on the scaffolds for 1 day

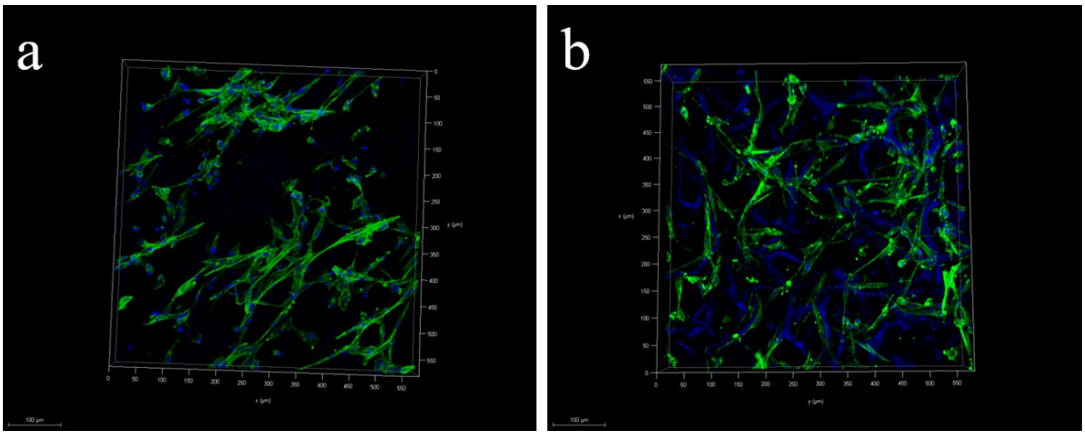

Figure S7. 2D structures of CLSM fluorescence micrographs of MSCs after 1 day normal culturing on PADM (a) and PADM-rGO (b), the actin filaments of the cells were stained by Alexa-fluor488-phalloidin with an excitation wavelength at 488 nm (green) and nuclear staining with DAPI (blue).

In the fluorescence micrographs, the cell quantities on PADM-rGO hybrid scaffold was almost as large as on the PADM scaffold after 1 day normal culturing.

S8. Cell proliferation assay

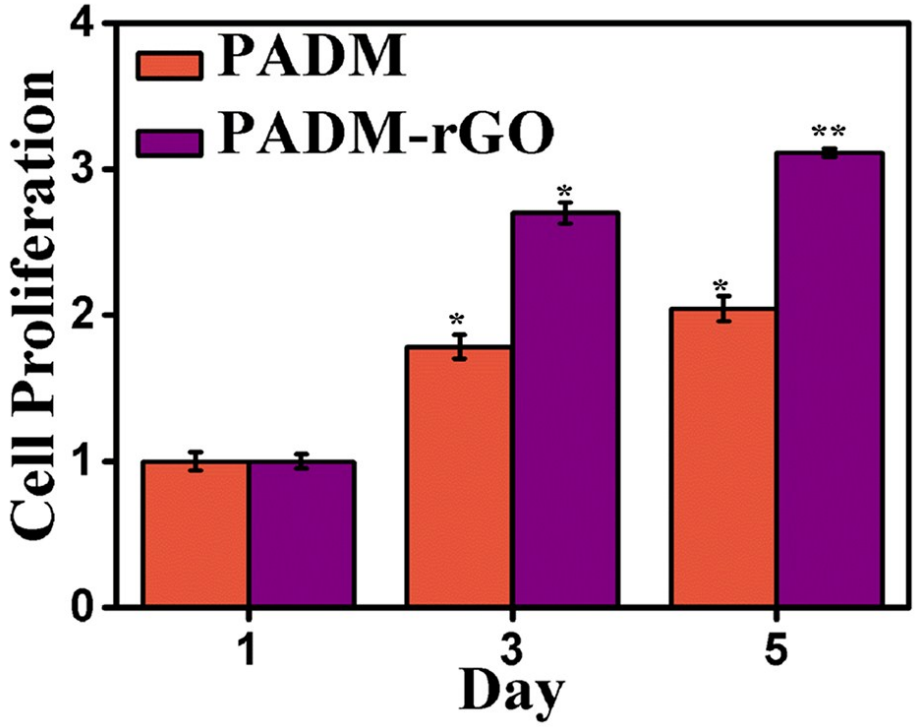

Figure S8. The proliferation of MSCs cultured on PADM and PADM-rGO scaffolds in 5 days (c) was

measured by CCK-8 assay. (*p ≤ 0.05, **p ≤ 0.01, n=3)

A Cell Count Kit-8 (CCK-8, Dojindo Molecular Technology) was used to quantitatively evaluate cell viability on PADM and PADM-rGO samples after cultivation for 1, 3 and 5 days. 2-(2-methoxy-4-nitrophenyl)-5-(2, 4-disulfophenyl)-2 H-tetrazolium monosodium salt (WST-8) was used as a substrate. First, each sample was moved to a new 48-well plate filled with 400 µL serum-free L-DMEM medium plus 40 µL CCK-8 solution per well. After 6 hours incubation at 37 °C, the resultant production of water soluble formazan dye was assayed at wavelength of 450 nm by a microplate reader (MULTISKAN MK3, Thermo, USA). Three parallel replicates for each sample were used.

S9. The ingrowth section images of MSCs on PADM and PADM-rGO scaffolds

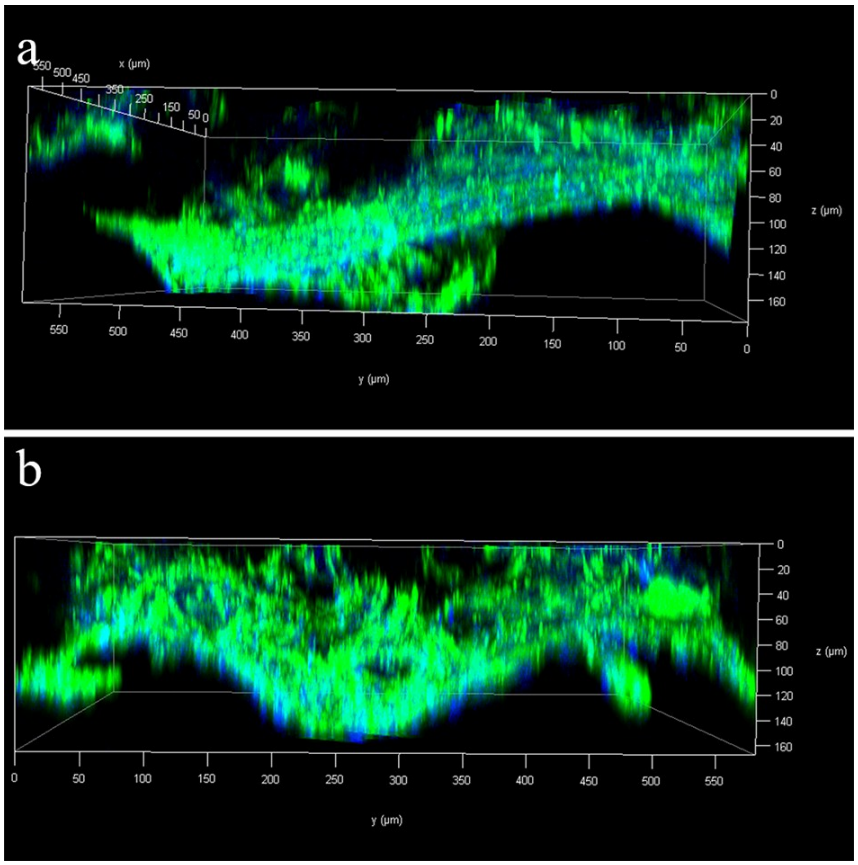

Figure S9. Typical 3D CLSM section images of the MSCs actin filaments on PADM (a) and PADM-rGO (b).

The typical 3D CLSM section images of the actin filaments demonstrated that the cells can migrate into the porous of PADM (a) and PADM-rGO (b) scaffolds and the depth of cells ingrowth can reach about 160 µm or 180 µm after 3 days culture.

S10. Sequences of Real-Time PCR primers

Gene	Forward primers (5'-3')	Reverse primers (5'-3')
GAPDH	GCCTCGTCTCATAGACAAGATGGT	GAAGGCAGCCCTGGTAACC
Nestin	AGAGTCAGATCGCTCAGATC	GCAGAGTCCTGTATGTAGCCAC
Tuj1	TAGACCCCAGCGGCAACTAT	GTTCCAGGCTCCAGGTCCACC
GFAP	CGGAGACGTATCACCTCTG	TGGAGGCGTCATTCGAGACAA
MAP2	GCCAGCATCAGAACAAACAG	AAGGTCTTGGGAGGGAAGAAC

Table S1. Sequences of Real-Time PCR primers

References

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2. W. S. Hummers Jr and R. E. Offeman, *Journal of the American Chemical Society*, 1958, 80, 1339-1339.
3. C. Cha, S. R. Shin, N. Annabi, M. R. Dokmeci and A. Khademhosseini, *ACS nano*, 2013, 7, 2891-2897.
4. D. R. Dreyer, S. Park, C. W. Bielawski and R. S. Ruoff, *Chemical Society Reviews*, 2010, 39, 228-240.