

Supporting Information

A novel zwitterionic hydrogel incorporated with graphene oxide for bone tissue engineering: Synthesis 、 characterization and promotion of osteogenic differentiation of bone mesenchymal stem cells

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1. Cell extraction, culture and differentiation of rBMSCs

The Sprague-Dawley (SD) rats used in this research were obtained from the Animal Experimental Center of Tongji University ((Shanghai, China). The rBMSCs were acquired as described in Reference¹, rBMSCs were isolated and cultured from 4-week-old Sprague-Dawley (SD) rats. All experiments followed the procedure according to the guidelines of the Animal Experimental Ethical Inspection of Tongji University School of Medicine and were approved by the Institutional Animal Care and Use Committee. The femurs and tibias were dissected, and bone marrow cavities were flushed with Phosphate Buffered Solution (PBS). rBMSCs (the bone marrow) were collected, purified by centrifugation, and then suspended in a 25 cm² flask containing the completed α - Minimum Essential Medium (α -MEM, Gibco, USA) with 1% penicillin/streptomycin (HyClone, USA) and 10% fetal bovine serum (FBS, Excell, Australia) and placed in an incubator at 37°C with 5% CO₂. The culture medium was refreshed every 2 days. The trypsinization and passage were performed when cells reached approximately 80% confluence. Passage 3-4 cells were used for subsequent *in vitro* experiments. For osteogenic differentiation, cells were influenced by osteogenic differentiation substrate, as cultured in DMEM supplemented with 50 mg/ml

L-ascorbic acid (Sigma-Aldrich, USA), 1% Pen-Strep, 10% FBS, 100 nM dexamethasone (Sigma-Aldrich, USA), and 10 mM glycerol-2-phosphate (Sigma-Aldrich, USA)². The osteogenic differentiation medium was replaced every day.

2. Supplementary tables and figures

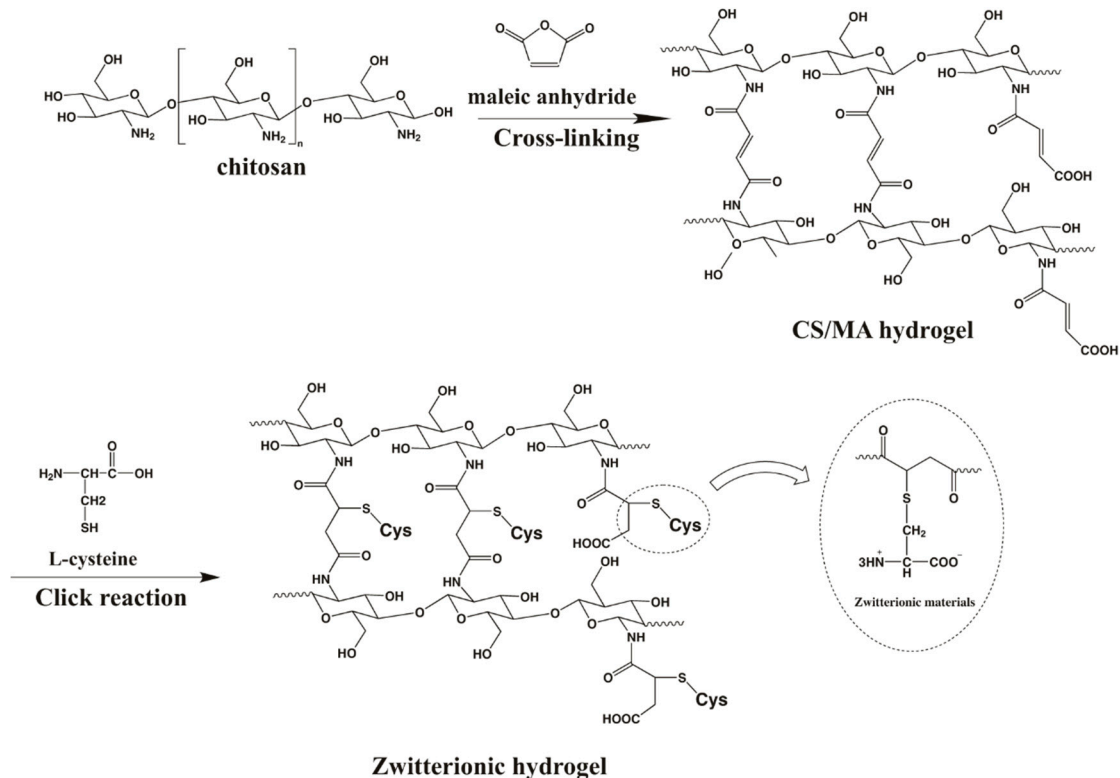


Figure S1. Synthesis of the zwitterionic hydrogel.

Table S1.

Sequences of primers used in the qRT-PCT	
Gene	forward and reverse primer sequences
RUNX2	F: 5'-CTTCGTCAGCGTCCTATCAGTTCC-3'
	R: 5'-TCCATCAGCGTCAACACCATCATTC-3'
OPN	F: 5'-GACGATGATGACGACGACGATGAC-3'
	R: 5'-GTGTGCTGGCAGTGAAGGACTC-3'
ALP	F: 5'-GGCGTCCATGAGCAGAACTACATC-3'
	R: 5'-CAGGCACAGTGGTCAAGGTTGG-3'
COL 1	F: 5'-TGTTGGTCCTGCTGGCAAGAATG-3'
	R: 5'-GTCACCTTGTCGCTGTCTCAC-3'
OCN	F: 5'-GGACCCTCTCTGCTCACTCTG-3'
	R: 5'-ACCTTACTGCCCTCCTGCTTGG-3'
β-catenin	F: 5'-ACAAGCCACAGGACTACAAGAAACG-3'
	R: 5'-TCAGCAGTCTCATTCCAAGCCATTG-3'
GAPDH	F: 5'-GACATGCCGCCTGGAGAAAC-3'
	R: 5'-AGCCCAGGATGCCCTTTAGT-3'

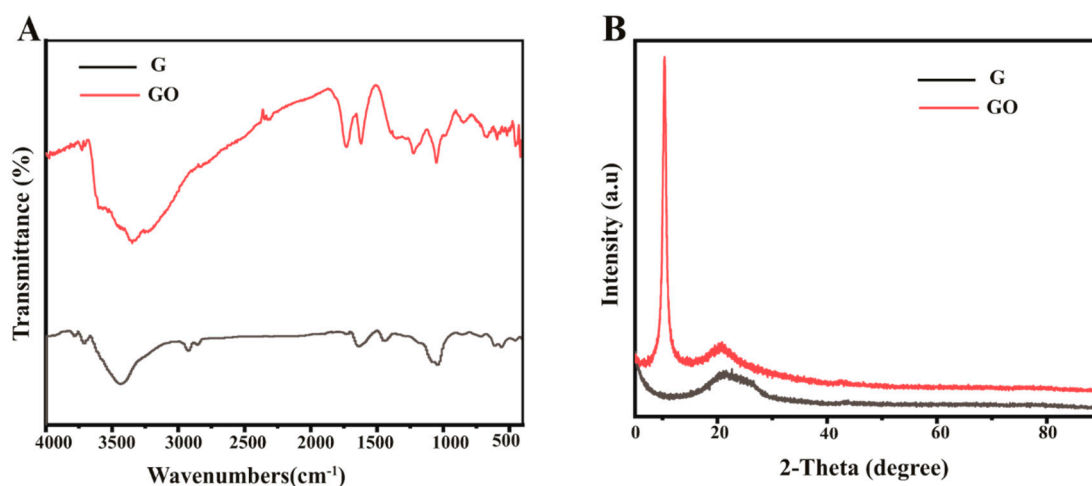


Figure S2. (A) FT-IR analysis and (B) XRD analysis of G and GO

3. surgical procedures

Eighteen male Sprague-Dawley rats of 10 weeks old were randomly divided into three groups: blank, Z-CS/ β -TCP and Z-CS/ β -TCP/GO-2 groups. The surgery followed these steps,³ after anesthetized by intraperitoneal injection with pentobarbital (4mg/100 g), two symmetrical full-thickness calvarial defects (diameters of 5 mm) were drilled by dental trephine. The scaffolds were randomly loaded in the calvarial defect while the group without any scaffolds implanted was the blank group. After implantation, the wounds were sutured by 4-0 silk sutures. At 6,12 weeks post-surgery, the rats were euthanized by injecting an overdose of pentobarbital.

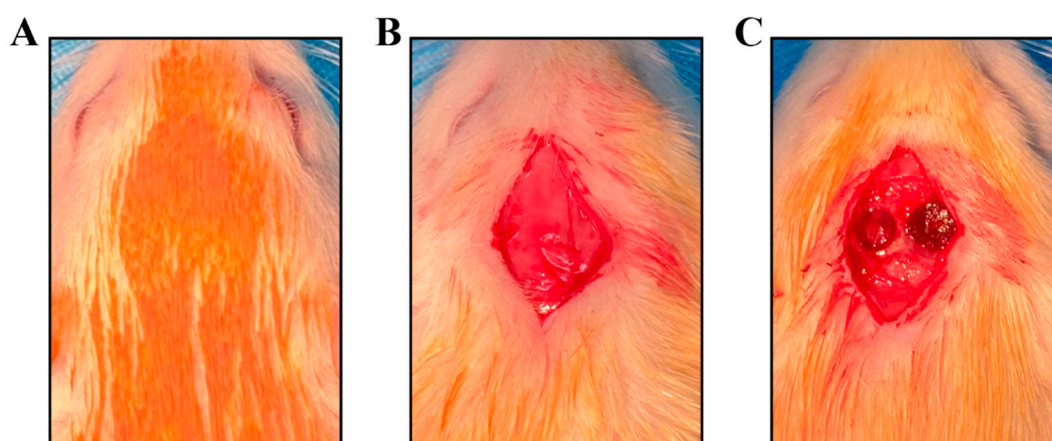


Figure S3. Surgical procedures:(A) disinfection,(B) skin incision,(C) load scaffold (right).

- 1 Yu, P., Bao, R. Y., Shi, X. J., Yang, W. & Yang, M. B. Self-assembled high-strength hydroxyapatite/graphene oxide/chitosan composite hydrogel for bone tissue engineering.

- Carbohydr Polym* **155**, 507-515, doi:10.1016/j.carbpol.2016.09.001 (2017).
- 2 Kim, J. *et al.* Enhanced osteogenic commitment of murine mesenchymal stem cells on graphene oxide substrate. *Biomater Res* **22**, 1, doi:10.1186/s40824-017-0112-8 (2018).
- 3 Chen, Y. *et al.* Developing a Strontium-Releasing Graphene Oxide-/Collagen-Based Organic-Inorganic Nanobiocomposite for Large Bone Defect Regeneration via MAPK Signaling Pathway. *ACS Appl Mater Interfaces* **11**, 15986-15997, doi:10.1021/acsami.8b22606 (2019).