Supplementary data

Mussel-inspired graphene oxide nanosheets enwrapped Ti scaffolds with drugs encapsulated gelatin microspheres for bone regeneration

Lu Han[1], Honglong Sun[1], Pengfei Li[1], Pengfei Tang[1], Chaoming Xie[1], Menghao Wang[1], Kefeng Wang[2], Jie Weng[1], Hui Tan[3], Fuzeng Ren[4], Xiong Lu[*1]

[1] Key Lab of Advanced Technologies of Materials, Ministry of Education, School of Materials Science and Engineering, Southwest Jiaotong University, Chengdu 610031, Sichuan, China

[2] National Engineering Research Center for Biomaterials, Genome Research Center for Biomaterials, Sichuan University, Chengdu 610064, Sichuan, China

[3] Shenzhen Key Laboratory of Neurosurgery, The First Affiliated Hospital of Shenzhen University, Shenzhen, Guangdong 518035, China

[4] Department of Materials Science and Engineering, South University of Science and Technology of China, Shenzhen, Guangdong 518055, China

* Corresponding Author

Tel.: +86-28-87634023

Fax: +86-28-87601371

Email address: luxiong_2004@163.com

Fabrication of GO

Briefly, 2 g of graphite powder was added to 46 ml of cold (~0 °C) H_2SO_4 in a flask. The mixture was stirred in an ice water bath for 30 min, and then 6 g of $KMnO_4$ was slowly added under vigorous stirring. The reaction was kept at a temperature lower than 20 °C for 2 h. Then, the ice water bath was removed, and the reaction mixture was maintained at 35 °C for 0.5 h. Subsequently, 30 ml of deionized water was gradually added into the mixture, and the reaction flask was transferred into a 98 °C water bath for 15 min. Finally, the mixture was washed with hydrochloride acid (5%) and deionized water several times to obtain GO.

Gelatin microspheres preparation

A 5 g aliquot of gelatin was dissolved in 45 ml of water and added dropwise to 200 ml olive oil to create a water-in-oil emulsion. The solution was stirred at 500 rpm and chilled to 10 °C for 1.5 h; microspheres were then collected by washing with acetone, followed by vacuum filtration. They were crosslinked overnight in a glutaraldehyde solution, and the reaction was terminated by the addition of glycine (25 mg/ml) to block residual aldehyde groups. The gelatin microspheres (GelMS) were again washed in acetone and collected by filtration, lyophilized and then sieved to obtain particles.

In vitro cell culture

Bone marrow stromal cells (BMSCs) were used to evaluate the *in vitro* behaviors of the scaffolds. Five types of scaffolds were used to culture BMSCs, namely, pure Ti scaffold, BMP2-Ti, GO/Ti, CGelMS-GO/Ti, and BMP2+Van+CGelMS-GO/Ti scaffolds. Briefly, third passage BMSCs (10^5 cells/sample) were cultured with different scaffolds to investigate their biocompatibility and osteoinductivity. The morphologies of BMSCs on the different scaffolds were observed using CLSM (TCSSP5) after stained by cell Live&dead staining (A017, GeneCopoeia Inc., USA) at the third day. The proliferation of BMSCs was evaluated using a MTT assay (Sigma, USA) after 3 and 7 d of culture. The differentiation of BMSC was determined using an alkaline phosphatase (ALP) kit (Nanjing Jiancheng Bioengineering Institute, China) after 14 d of culture. There were 5 parallel samples for each scaffold.

Antibacterial activity test

Four kinds of scaffolds, namely, Ti, GO/Ti, CGelMS-Ti, and BMP2+Van+CGelMS-GO/Ti scaffolds were used for the test. Briefly, 400 μl of bacteria suspension (1×10^6 CFU/ml) was added onto the scaffold samples. After 4 h, 600 μl of Luria-Bertani broth was added. Then the samples were placed in an incubator at 37 °C with constant agitation. After 1 d, 150 μl of bacterial suspension was collected and the optical density (OD) of the suspension at 600 nm was measured using a micro plate reader (MQX200).

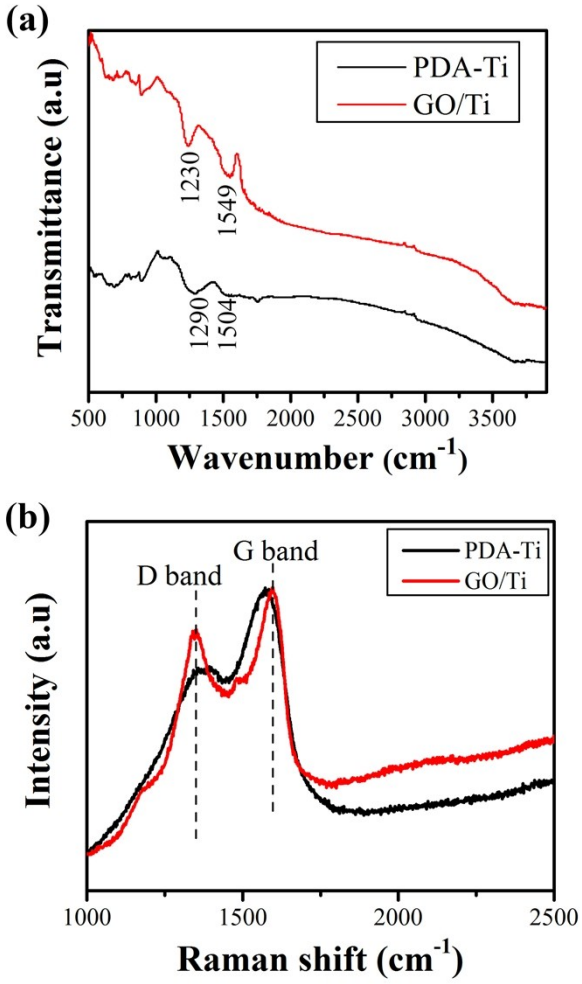

Figure S1. (a) FTIR and (b) Raman spectra of PDA-Ti and GO/Ti scaffolds.