Additional file 1 The physical and chemical properties of CS-GO scaffolds

Materials and reagents

Chitosan (> 85% deacetylated; Aoxin Biotec, Taizhou, China) and graphene oxide (diameter 0.5-3 μm, thickness 0.55-1.2 nm; Time Nano, Chengdu, China) and genipin (Zhi Xin Biotechnology, Wuzhou, China) were used to fabricate scaffold. Dulbecco's modified Eafle's medium (DMEM; Gibco, Grand Island, NY, USA), Heat-inactivated fetal bovine serum (FBS; Bovogen, Melbourne, Australia), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma, Santa Clara, CA, USA) were used for cell culture and toxicity test. Goat serum (Boster, Pleasanton, CA, USA), paraformaldehyde from Boster, chloral hydrate from Sigma, and pentobarbital sodium from Sigma were used for animal surgery and test.

Microstructure observation

The microstructure of CS-GO composite scaffolds was observed using scanning electron microscope (SEM; S4800; Hitachi, Tokyo, Japan). CS-GO scaffolds were cut into cross-section, and sputter-coated with a layer of gold and observed under SEM with an accelerating voltage of 5 kV. The diameters of micro-pores were measured, based on SEM images with higher magnification.

Composition analyses of CS-GO scaffolds

Fourier transform infrared (FT-IR, Nicolet 5700, Madison, WI, USA) patterns of CS and CS-GO samples were recorded in the wavenumber range from 400 to 4000 cm^{-1}; Raman analysis of two samples was performed using Raman spectroscopy (Thermal Scientific DXR, Waltham, MA, USA) equipped with 514 nm laser and Leica microscopy; The scaffolds were immersed in phosphate-buffered saline for 30 minutes, and elastic modulus of scaffolds was tested using a single fiber tensile machine (YG005A; Baien Instrument, Wenzhou, China); Four probe record (RTS-9; PROBES TECH, Guangzhou, China) was used to measure the electrical conductivity of pure CS film, dry CS-GO film, and CS-GO film immersed in phosphate-buffered saline for 0, 7 and 14 days.

Biodegradation of GO by horseradish peroxidase

Mixtures of 300 × *g* of GO sample and 150 × *g* of horseradish peroxidase (Cat# P8020; Solarbio

Science & Technology, Beijing, China) were evenly dispersed in 1 mL of 0.05 M of phosphate buffer containing 0.14 M of NaCl. H_2O_2 was added at a rate of 0.2 M/h. Horseradish peroxidase was renewed every 5 hours (namely at 0, 5, 10, 15, and 20 hours). The reaction mixture was maintained at 37^{o}C for 24 hours. The control experiment for assessing the degradation of GO sample by only adding equivalent phosphate-buffered saline was also performed using the same protocol.

Transmission electron microscopy (Tecnai G2 F20 S-TWIN, FEI, Hillsboro, OR, USA) characterization was performed for two samples after degradation experiment. Dynamic light scattering (Zetasizer Nano, Malvern, UK) was performed simultaneously to analyze the size change of GO sheets to characterize their degradation from horseradish peroxidase.