Electronic Supplementary Material (ESI) for RSC Advances.
This journal is © The Royal Society of Chemistry 2015

# Supplementary Information

# Starch-borate-graphene oxide nanocomposites as highly efficient targeted antitumor drugs

Rumei Cheng[a], Shengju Ou[b], Yexu Bu[a], Xuan Li[a], Xiaohong Liu[a], Yuqin Wang[a], Rui Guo[a],Bingyang Shi[c], Dayong Jin[c,d*], and Yong Liu[a,c*]

[a]*Institute of Advanced Materials for Nano-Bio Applications, School of Ophthalmology & Optometry, Wenzhou Medical University, Wenzhou, Zhejiang 325027, China*
[b]*Nanjing Landa Femtosecond Insepection Techonlogy Co. Ltd., Nanjing High-tech Industry Development Zone, Nanjing, Jiangsu 210032, China*
[c]*Advanced Cytometry Labs, ARC Center of Excellence for Nanoscale BioPhotonics, Department of Chemistry and Biomolecular Science, Macquarie University, Sydney, NSW 2109, Australia*
[d]*Institute for Biomedical Materials and Devices, Faculty of Science, University of Technology Sydney, NSW 2007, Australia*
Emails: yongliu1980@hotmail.com; dayong.jin@uts.edu.au

## Experimental section

### Chemicals

Graphite with an average particle size of 100 μm was obtained from Shanghai Reagent Co., Ltd. GO was prepared via the Hummers' method.[1] Soluble starch, boric acid, and sodium borate were purchased from Aldrich Co., Ltd. All other reagents and chemicals were of analytical grade and used as received. All solutions were prepared using doubly distilled Millipore water filtered via a 0.45 μm membrane.

### Synthesis of starch-borate-GO nanocomposites

Dried soluble starch was suspended in 100 mL of sodium hydroxide aqueous solution. The mixture was heated at 60 °C until a transparent solution was obtained. Meanwhile, boric acid aqueous solution was added dropwise under magnetic stirring. The reaction mixture was additionally stirred for several hours at 60 °C. 0.1 mg/mL GO suspension was subsequently added into the starch complex solution above. The mixture was then stirred at 200 rpm at room temperature overnight and a precipitation was consequently collected and washed by distilled water. The as-prepared materials were dried in a vacuum oven at 50℃ for 24 h and kept in a desiccator. Nanocomposites with different constituent proportion ($n_{starch}$: $n_{borate}$: $n_{GO}$ =1:1:1; 1:2:1; 1:3:1; 2:1:1; and 3:1:1) were prepared and designated as S1, S2, S3, S4, and S5 respectively throughout the article. The resulting SBG was obtained after drying at room temperature and followed by further drying in a vacuum oven at 50 °C for 24 h. The loading content of borate in each sample was determined by the ion chromatography. Percentage of loaded borate was found to be 7.21%, 13.36%, 17.68%, 10.41%, and 11.25%, respectively.

### Characterisation

Morphology of SBG was observed using a TEM (Hitachi H-9000NAR) with an accelerating voltage of 100 kV. FT-IR was recorded with a PE Spectrum One spectrometer using KBr pellets in the 4000–450 $cm^{-1}$ region. XPS (Perkin-Elmer PHI-5300 ESCA) was used for surface chemical analysis. TGA was performed using a STA 409 PC/4/H Lux at a heating rate of 10 ℃ per minute under $N_2$. Raman spectra were determined using a Jobin spectrometer with an excitation wavelength of 532 nm. The ion chromatography was carried out using ThermoFischer ICS-900.

**Cell culture**

The cells were cultured as we reported elsewhere.[2] ARPE-19 (a cell line derived from human RPE) cells were cultured in Dulbecco's modified Eagle's medium/F12 (12800-017, high glucose Gibco), supplemented with 10% fetal bovine serum (SV 30087.02, Hyclone) and 50 μg/mL gentamicin. OCM-1 (a cell line from human CM) cells were cultured in RPMI 1640 medium with 10% fetal bovine serum (SV 30087.02, Hyclone) and 50 μg/mL gentamicin. Both were incubated in a humidified 5% $CO_2$ balanced air incubator at 37℃. Medium was changed every 2 days. The cells were passaged with 0.25% trypsin (Invitrogen) plus 0.02% EDTA (Sigma). The sample without addition of nanomaterials was used as the control.

**The CCK-8 assay**

Cell viability was measured using CCK-8 assays as reported elsewhere. All samples were cut into 0.5 × 0.5 cm blocks, sterilized in absolute ethanol overnight, and then rinsed with PBS solution three times. 5000 cells in 100 μL of medium were seeded into each well of 96-well culture plates. Sterilized samples were incubated with cells for 24 or 48 h, respectively. 10 μL CCK-8 solution was added to each well and incubated for 3 h. Absorbance was measured at the wavelength of 450 nm using a microplate reader (Biorad 680).

**Statistical analysis**

Statistical analysis was performed using SPSS 18.0. All data were expressed as the mean ± SD. Statistical comparisons of the means were carried out via two-way analysis of variance (ANOVA) with Bonferroni post-tests. The significance level was set at $p < 0.05$.

## Results

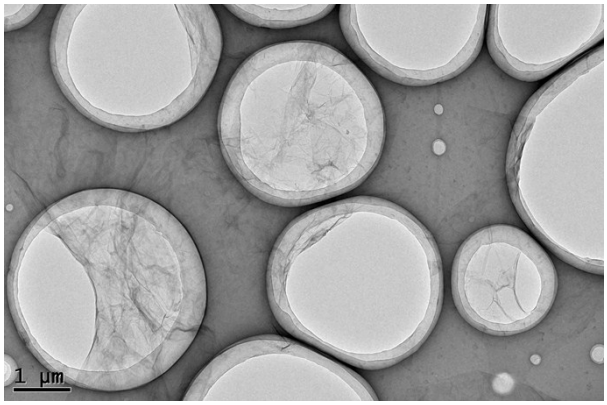


Figure S1. TEM image of GO.

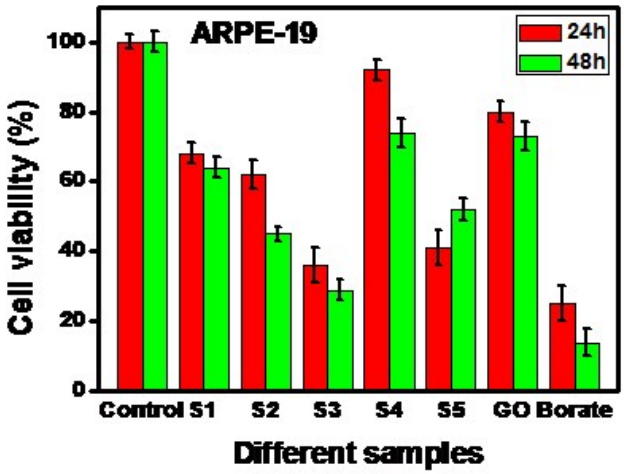


Figure S2. Cell viability of different samples with ARPE-19 cells. S1 to S5 are the SBG samples prepared from $n_{starch}$:$n_{borate}$:$n_{GO}$ =1:1:1; 1:2:1; 1:3:1; 2:1:1; 3:1:1, respectively. Cell culture time: 24 h and 48 h. The concentration of all samples is 100 μg/mL. The sample without addition of nanomaterials was used as the control.

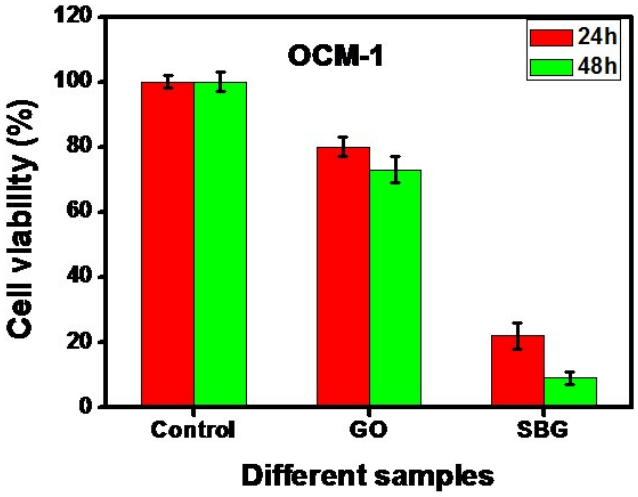


Figure S3. Cytotoxicity of GO and starch-borate-GO (at the ratio 2:1:1, S4) nanocomposite against OCM-1 cells after incubation for 24h and 48h. The concentration of all samples is 100 μg/mL. The sample without addition of nanomaterials was used as the control.

## Reference

1. W. S. Jr. Hummers and R. E. Offeman, *J. Am. Chem. Soc.*, 1958, 80, 1339.
2. M. Lin, R. Zou, H. Shi, S. Yu, X. Li, R. Guo, L.Yan, G. Li, Y. Liu, and L. Dai, *Mater. Sci. Eng. C* 2015, 50, 300.