Supporting Information

Impact of graphene exposure on microbial activity and community ecosystem in saliva

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Experiment section

Materials.

Graphite powder, sulfuric acid, potassium permanganate, and other chemical reagents by used to synthesize GO, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) all were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium nitrate and silver nitrate were purchased from Sinopharm Chemical Reagent (China). Propidium monoazide (PMA) was obtained from Geneasy, China. Brain heart infusion broth (BHI), Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS) and Live/Dead BacLight Bacterial Viability Kits were purchased from Invitrogen Ltd., UK. All the chemicals used in this research were research grade, and all aqueous solutions were prepared with deionized (DI) water from Milli-Q-Water (Millipore Corp, 18.2 MΩ/cm at 25 °C).

Effect of GMs on salivary bacterial community

The cultured salivary microbial suspension (OD_{600}=0.6, 200 uL) was prepared in the tube. Then, different concentrations of GMs (50 uL) were added to the tubes. The final concentration of GO-AgNPs were 2.5, 5, 10, 15, 20 and 40 μg/mL respectively, and of GO were 20, 40, 80, 120 and 160 μg/mL respectively. The saline solution was served as control. All bacteria were grown at 37 °C in an anaerobic system (80% N_2; 10% H_2; 10% CO_2). After 2 hours, the microbial activity and community ecosystem in saliva was investigated by various assays.

MTT Assay: The 50 μL of MTT was added into each tube after 2-hour incubation, and these tubes were stored in dark for another 2 h. Then, the solutions were centrifuged at 5000 rpm for 5 min and discarded the supernatant. Dimethyl sulfoxide (250 μL) was used to dissolve bacterial cells in the bottom of the tubes. The absorbance of bacterial lysis solution was measured at 490 nm using microplate reader (Bio-Rad, USA). The bacterial viability was expressed as a percentage of $OD_{test}/OD_{control}$. All of the assessment data were performed in triplicate, and at least three independent experiments were done.

Live/Dead bacteria Assay: Live/Dead BacLight Bacterial Viability Kits was used to distinguish the live and dead bacteria, which was stained by green fluorescent SYTO 9 and red fluorescent propidium iodide (PI) respectively. The bacteria exposure to GMs (250 μL, 20 μg/mL GO-AgNPs and 80 μg/mL GO, respectively) were stained with 1.5 μL the dye in the dark at room temperature for 15 min. Then, flow cytometry (BD Biosciences, USA) was used to calculate the number of live and dead bacteria. Run each sample and acquire data for at least 10000 events. And confocal laser scanning microscopy (CLSM, Leica TCS SP2, Germany) was carried out the observation of bacterial morphology.[37-39] SYTO 9 and PI fluorophores were excited at 488 nm, and the emissions were collected at 500-550 nm and 590-680 nm respectively.

Transmission Electron Microscopy: After exposure to GMs (20 μg/mL GO-AgNPs and 80 μg/mL GO, respectively), bacterial communities were fixed with 2% glutaraldehyde for 2 h at 4 °C, washed with PBS, and then fixed with 1% aqueous OsO_4 solution for 2 h. After washed with PBS, the dehydration steps were performed with 30% and 50% ethanol for 10 min, 70% ethanol for 18h. Then 80%, 95% and 100% ethanol for 10 min. And then the mixture was embedded with Epon/Araldite resin at 60 °C for 48 h. Finally, these resins were cut into ultrathin sections (90 nm) by ultra-microtome and stained with 4% uranyl acetate (1:1 acetone/water) and 0.2% lead citrate for 1 min. Samples were then well prepared and examined under the transmission electron microscopy (PHILIP CM-120).

PMA treatment

Bacterial community was exposed to GMs (20 μg/mL GO-AgNPs and 80 μg/mL GO, respectively) for 2 hours, and the mixtures were centrifuged at 5000 rpm for 5 min, the supernatant was discarded. The precipitates were then washed one time and resuspended in PBS. The optical density of the

bacterial suspensions at 600 was adjusted to 0.6, and PMA with a final concentration of 50 μM was added. After mixed thoroughly, the mixtures were put into LED Photoactivation Device (Geneasy, China) for 10 min, for dark blending at room temperature. Turn on the LED light for another 10 min. Then the tubes were centrifuged at 10000 rpm for 10 min, the supernatant was discarded. The precipitate was store at -80 °C until further use.

Cell lines and cell culture

All the cell lines were purchased from the SIBCB (Shanghai Institution of Biology and Cell Biology). NIH-3T3 mouse fibroblasts cells and Human embryonic kidney (HEK) 293T cells were both grown in DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin.

Cytotoxicity analysis.

The cells were seeded in 96-well plates (HEK 293T cells with 5×10^4 per well; NIH-3T3 cells with 1×10^4 per well), and different concentrations of GMs were added to each well after cells incubated overnight. Then the samples were incubated for 24 h at 37 °C under the humidified atmosphere (5% CO_2). MTT assays were performed to assess the metabolic activity of cells treated as described above. 10 μL stock MTT (5 mg/ml) was added to each well, and cells were then incubated for 4 h at 37 °C. Cells were lysed with acidulated SDS. Absorbance was measured at 570 nm using microplate reader (Bio-Rad 680, USA). The cell viabilities were expressed as a percentage of $OD_{test}/OD_{control}$. All of the viability assessment data were done in triplicate, and at least three independent experiments were performed.

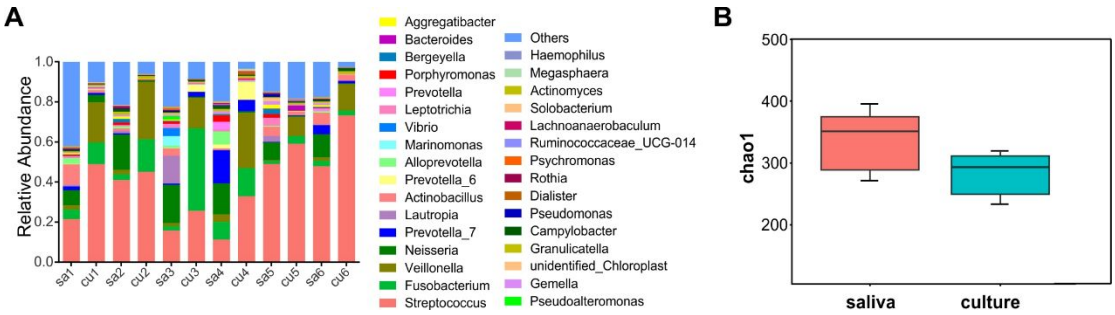

Figure S1 Comparison of the bacterial diversity of saliva and cultured saliva in six samples. (A) Stacked bar plot of phylogenetic composition of bacterial taxa (>0.5% abundance) at the genus level in salivary and cultured samples. Six samples are labeled with the number 1, 2, 3, 4, 5 and 6. (B) Chao 1 diversity scores of the microbiome in salivary and cultured samples.

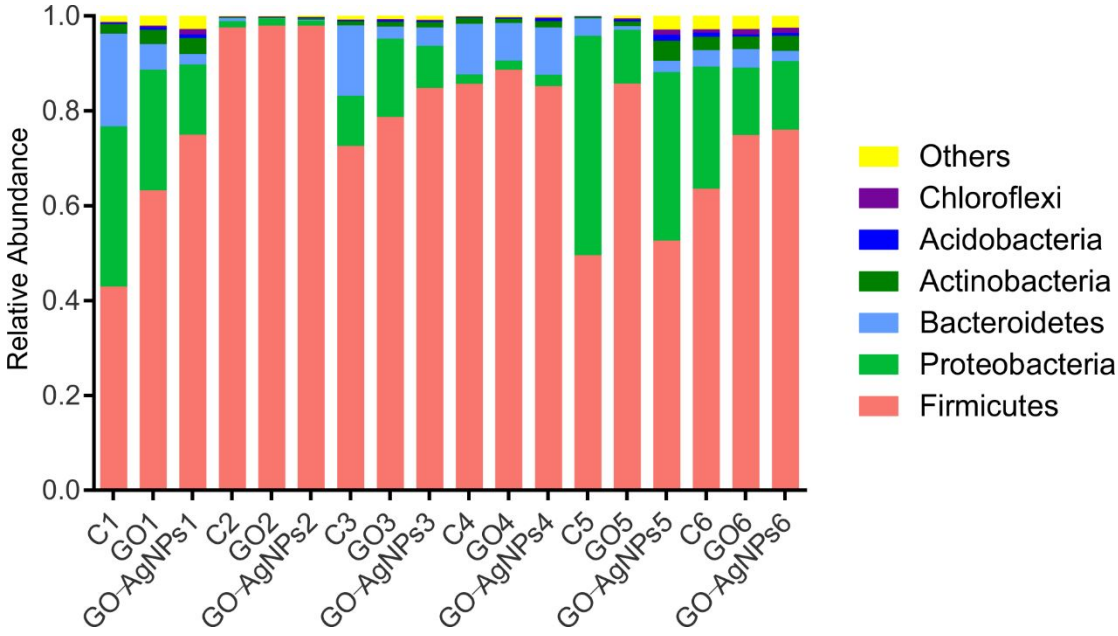

Figure S2 Stacked bar plot of phylogenetic composition of salivary bacterial taxa (>0.5% abundance) at the phylum level in control, GO and GO-AgNPs samples. Six samples are labeled with the number 1, 2, 3, 4, 5 and 6.

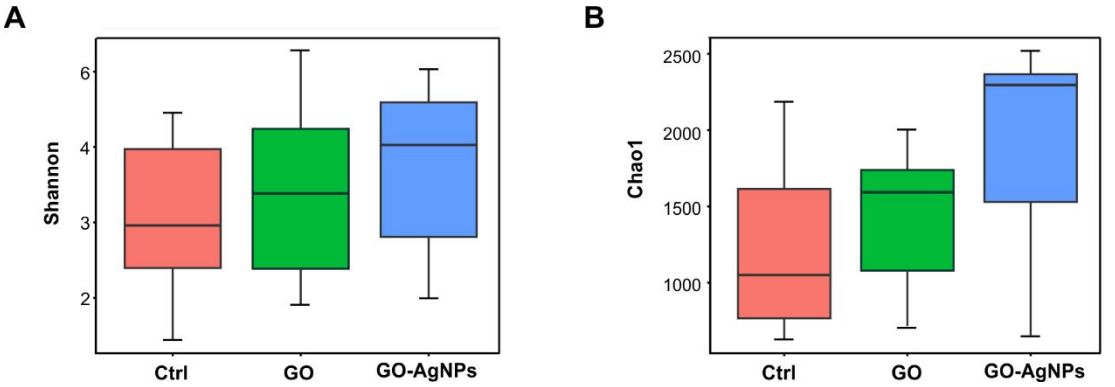

Figure S3 (A) Shannon diversity scores and (B) Chao 1 diversity scores of the microbiome in three groups, control, GO and GO-AgNPs.

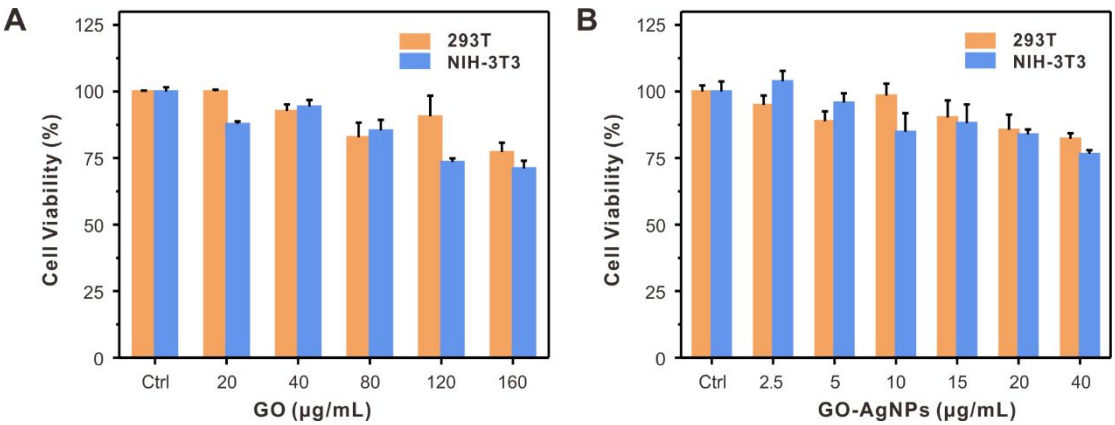

Figure S4. Cell viability studies in mammalian cells incubated with GMs. HEK 293T (orange) and NIH-3T3 cells (blue) were treated with different concentrations of (A) GO and (B) GO-AgNPs for 24 h, respectively.