Supplementary Materials

Carboxylated gold nanoparticles inhibit bone erosion through disturbing acidification of osteoclast absorption microenvironment

Materials and methods:

Cytotoxicity test

Cell Counting - 8 Kit and Annexin V-FITC/PI Kit (Dojindo Laboratories, Japan) were used to measure the cell viability and apoptosis according to the manufacturer's instruction. For cell viability test, the treated cell on 96-well plates were co-cultured in medium with 10% CCK-8 solution for 40 min and measured he absorbance at wavelength 450 nm using a microplate reader (Molecular Devices, USA). For cell apoptosis test, the treated were stained by PI/Annexin V-FITC for 15 min and measured the apoptosis ratio by flow cytometry (Bio-Rad, USA).

Gold content in cell test

The cells are exposed to AuNPs for 24h and collected by 0.25%tyrisin. The cells were rinsed by PBS for 3 times and remove PBS as much as possible. The rinsed cell dissolved in 30% hydrogen peroxide to degrade the cell and freeze-drying. The dried sample were dissolved in nitrohydrochloric acid for 18h, and redissolved in 0.2%Hcl and 0.1% HNO_3.

Rhodamine phalloidin staining

After successful induction of osteoclasts, they were fixed with 4% paraformaldehyde for 20min at room temperature. Remove paraformaldehyde and wash with PBS. Permeabilize cells with 0.5% Triton X-100 in PBS at room temperature for 3 mins. Block non-specific binding using 3% non-fat dry milk in PBS at 4℃ for 1 hour. Staining with 100Nm rhodamine phalloidin for 20 mins at room temperature. Washed the sample with PBS for 3 times, every time is 10 mins. Finally staining the nucleus with Hoechst33342.

Results:

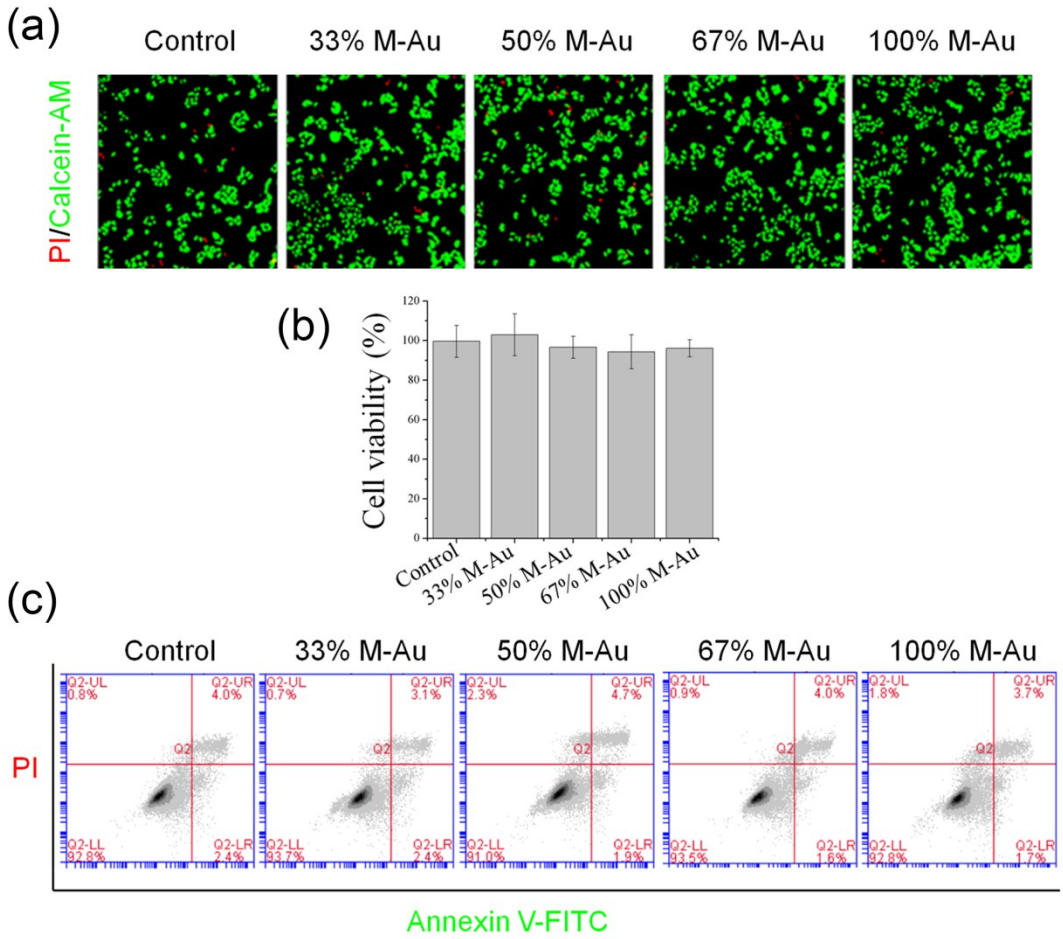

Figure S1: Cytotoxicity test of AuNPs. (a): Representative image of Calcein-AM/PI staining; (b): Cell viability determined by CCK-8; (c): apoptosis rate determined by Flow Cytometer.

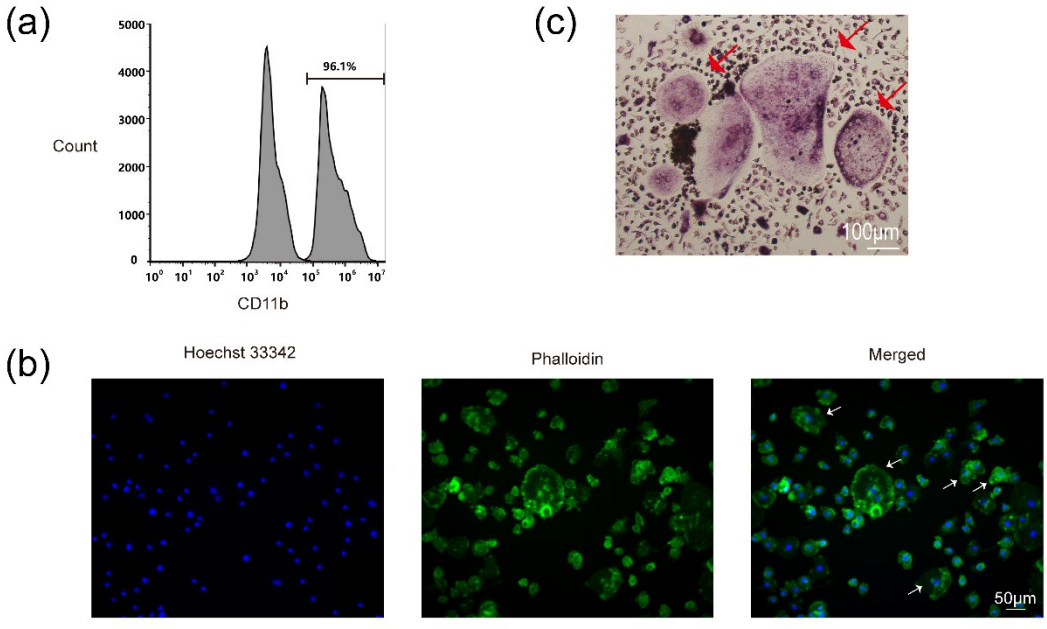

Figure S2: Cell type identification. (a): Bone marrow mononuclear cells (BMMCs) were identified by CD11b through Flow Cytometer; (b): Preosteoclasts were identified by the formation of multicellular cells; (c): Mature osteoclasts were identified by TRAP staining.

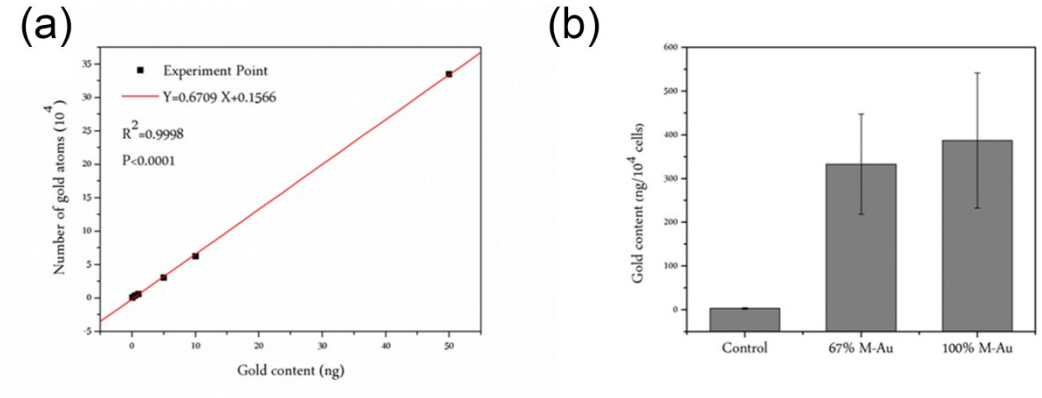

Figure S3: AuNPs internalized by cell. (a): Standard curve of gold nanoparticle concentration; (b): Gold content in Raw 264.7 cell after incubated with AuNPs for 24 h.

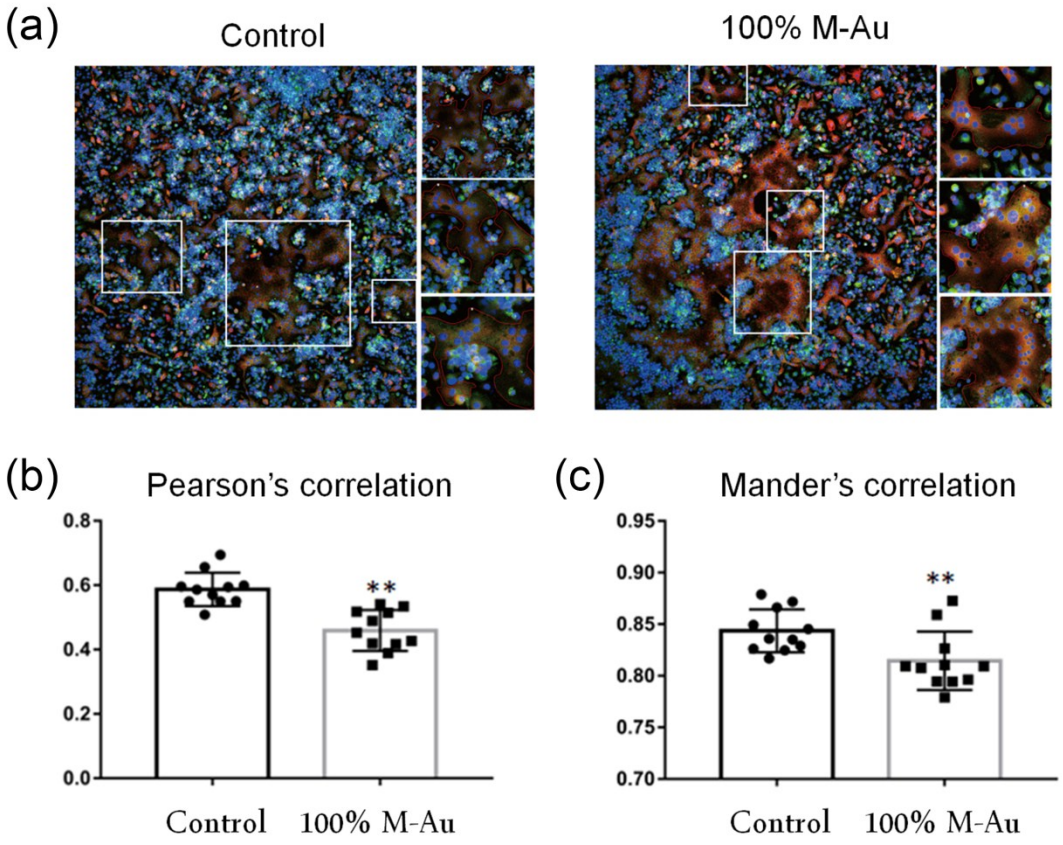

Figure S4: AuNPs decreased V-ATPase activity. (a): Representative fluorescent images of the colocalization area of V1 and V0; (b, c): The co-localization rate from three independent experiments; 30 cells were selected in each experiment.

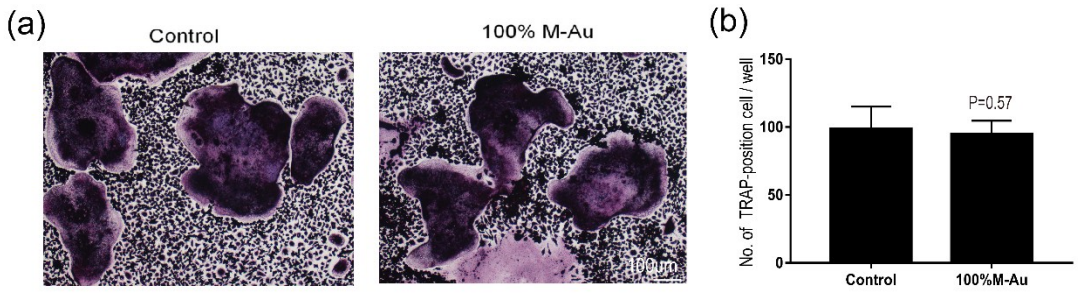

Figure S5: The effect of AuNPs on the formation of OCs. (a): Representative osteoclasts were stained by TRAP; (b): The number of OCs from three independent experiments; 4 views were selected in each experiment.

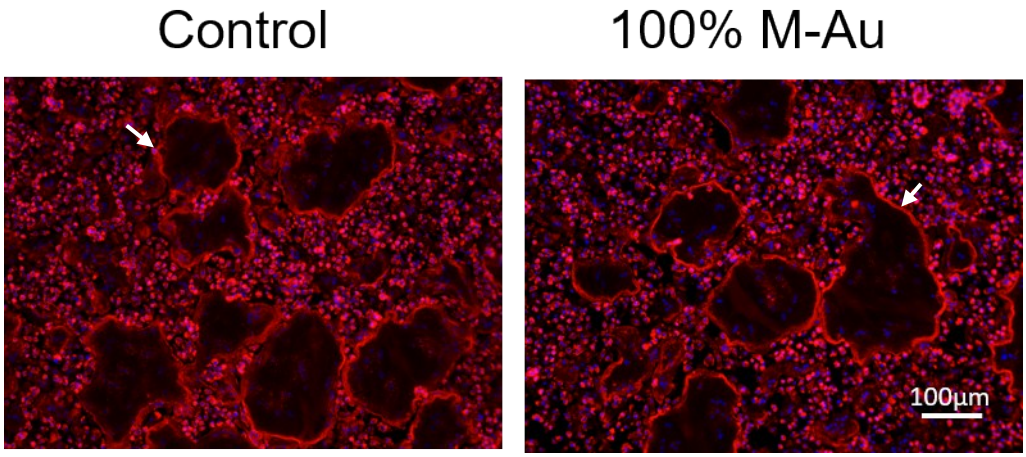

Figure S6: The effect of AuNPs on the sealing zone of OCs. OCs are induced from bone marrow cells on the glass bottom plate. The white arrow indicates the sealing zone.

Table S1 Acronym list

Abbreviation	Full name
AuNPs	Gold nanoparticles
OCs	Osteoclasts
V-ATPase	Vacuolar-type H^+-ATPase
OP	Osteoporosis
LPS	Lipopolysaccharide
TEM	Transmission electron microscopy
MUA	Mercaptoundecanoic acid
OT	Octanethiol
SPR	Surface plasma resonance
SEM	Standard error of the mean
MFI	Mean fluorescence intensity
BV	Bone volume
TV	Tissue volume
BS/TV	Bone surface density
Tb. N	trabecular number
Tb. Th	Trabecular thickness
Tb. Sp	trabecular separation
FLIM	Fluorescence life time imaging microscopy
ddH2O	Double distilled H_2O
UV-Vis	Ultraviolet and visible spectrophotometer
DLS	Dynamic laser scattering
EDS	Energy dispersive spectroscopy
FBS	Fetal bovine serum
BMMs	Bone marrow macrophages
α-MEM	α-minimum essential medium
M-CSF	Macrophage colony-stimulating factor
RANKL	Receptor activator of NF-κB
LSCM	Laser scanning confocal microscopy
TRAP	Tartrate-resistant acid phosphatase
CT	Computed tomography