Supplementary Material:

Autophagy in resin monomer-initiated toxicity of dental mesenchymal cells: a novel therapeutic target by N-acetyl cysteine

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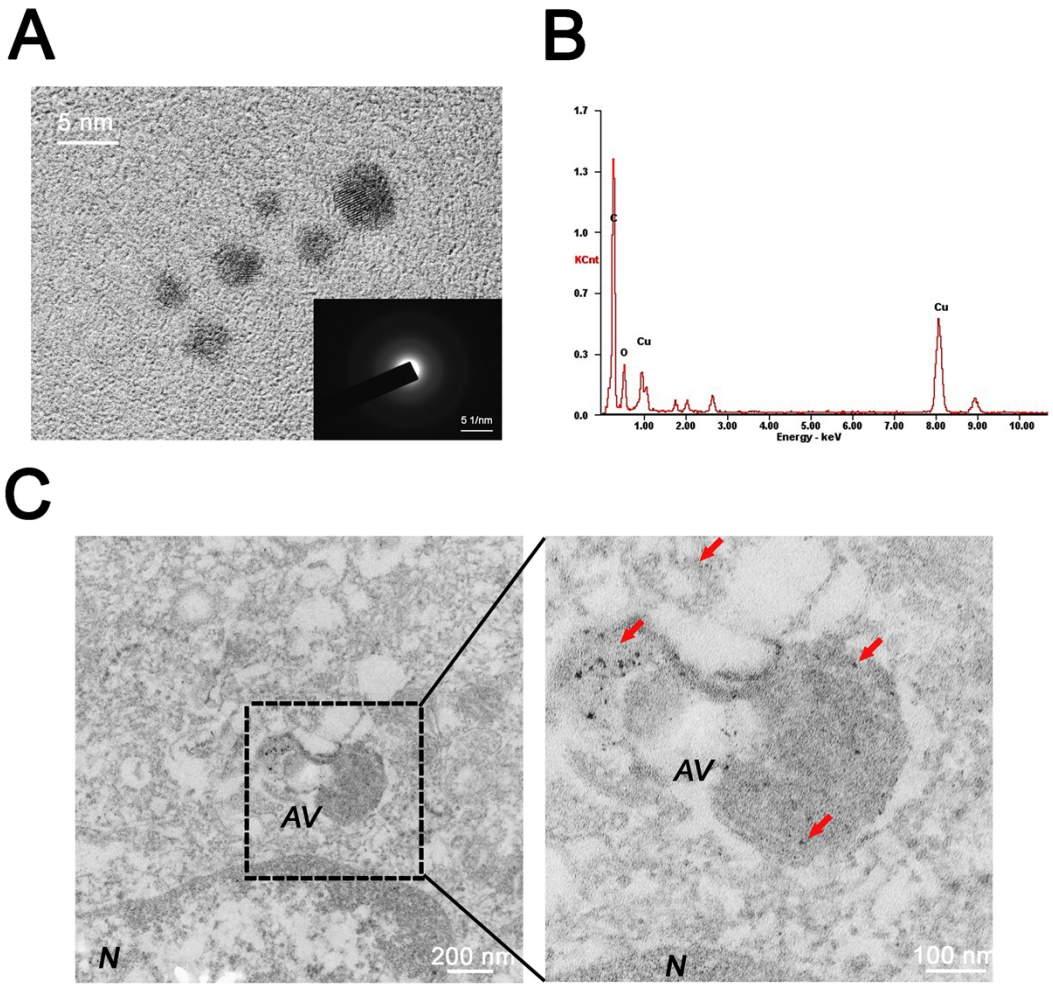
1 Supplementary Materials and methods

1.1 Transmission electron microscopy (TEM) analysis of TEGDMA nanoparticles

To detect the possible self-assembled nanoparticles from TEGDMA and measure the particle size, the original TEGDMA was suspended in PBS at 3 mM and sonicated for 5 min. Then a drop from the suspension was dropped over the copper-coated carbon grids. Grids were properly dried and examined under a Hitachi H-600 Transmission Electron Microscope. Selected area electron diffraction (SAED) patterns were also taken using this instrument. The composition of the self-assembled nanoparticles was determined by electron dispersive X-ray (EDX) analysis to exclude chemical contamination.

1.2 Bio-TEM Observation of TEGDMA-treated human dental mesenchymal cells (DMCs)

Bio-TEM was carried out to determine the possible consumption of self-assembled nanoparticles in TEGDMA-treated DMCs. Briefly, DMCs were seeded in 6-well plates and incubated with 3 mM TEGDMA for 6 h. Cells were washed with PBS and fixed overnight using 2.5% glutaraldehyde. This was followed by secondary fixation with 1% osmium tetraoxide, then dehydration in a gradient ethanol series. Embedded samples were sectioned and examined under a Hitachi H-600 Transmission Electron Microscope. Uranyl acetate staining was not used in this study to avoid uranyl acetate precipitation interfering with the TEM observation.



Supplementary Fig. 1 TEGDMA partially self-assembled into nanoparticles and entered into DMCs accompanied by autophagosomes. TEM and SAED observation (A), as well as EDX analysis (B) of self-assembled TEGDMA nanoparticles. (C) Detection of TEGDMA nanoparticles consumption and autophagic vacuoles in DMCs. *AV*, autophagic vacuoles; *N*, nuclear.