Enhanced X-ray Irradiation Induced Cancer Cell Damage by PEG-gold Nanoparticles

We demonstrate that pegylated Au nanoparticles synthesized by a novel X-ray irradiation method with size of 4.7±2.6 nm enhances the radiation response on CT26 cells. Substantial amount of pegylated gold nanoparticles were found uptaken by CT26 cells by TEM and confocal microscopy. These nanoparticles shows high biocompatibility and their internalization enhanced the cell damage induced by X-ray irradiation. Synchrotron Radiation based Fourier Transform Infra-Red spectromicroscopy was able to identify spectral features which can be linked directly to the cell damage. Specifically, the appearance of C=O stretching bonds shown in the spectral features were correlated to CT26 cell viability data, and therefore can be used as a biochemical marker for cell damage.

Beamlines

01A1 SWLS-White X-ray

14A1 BM-IR Microscopy

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Radiotherapy is one the primary and most effective means of curative treatment of malignant tumor. Substantial dose enhancement by the differential uptake of nanoparticles was reported. However, a clear chemical picture in cell level is still lacking. We targeted this issue by using a popular nanoparticle system, the PEG modified Au, in the form of colloidal solution, and studied in detail its uptake and radiotherapeutical response to CT26 tumor cells. Cytotoxicity tests, electron and optical microscopies and synchrotron radiation (SR) based Fourier-Transform infraRed spectromicroscopy measurements revealed that substantial amount of PEG-Au nanoparticles prepared by a SR synthesis method were internalized in cytoplasm of CT26 cells and subsquently enhanced the cell damage with X-ray irradiation.

The nanoparticle system was obtained with a recently developed synthesis procedure: irradiation by synchrotron X-rays produces colloidal solutions of gold nanoparticles with rather interesting properties compared to more conventional approaches. The process exploits the reduction due to the radiolytic effects of synchrotron X-rays and requires no reductant and no stabilizer in the precursor solution – therefore, the particle surfaces are immune from possible contaminations. The high-intensity synchrotron X-rays trigger a fast radiolytic reaction and yield a large quantity of nanoparticles in a clean, well-dispersed colloidal solution, stable and without the common problem of agglomeration in cell culture media. This approach could be easily extended to the preparation of PEG modified colloidal gold by simply adding the PEG into the precursor solutions and performing a one-solution X-ray irradiation synthesis. The obtained highly concentrated and stable pegylated gold colloidals enabled us to study the interaction of pegylated gold nanoparticles with cancer cells under realistic therapy-like concentrations and conditions.

Previous study indicated that the size of nanoparticles under 100 nm would be easily uptaken by cell. The

internalization of nanoparticles within cells might be an important factor which can be attributed to the enhancement of therapeutic effects. The presence of higher absorbing elements reside in the cells would increase the production of photoelectrons or free radicals and damage the cell organelle or nucleus. In our case, the confocal microscopy images and the TEM micrographs of CT26 cells treated with PEG modified gold nanoparticls clearly show that a substantial amount of PEG-Au nanoparticles were internalized. As shown in Fig. 1, PEG-Au nanoparticles inside vesicles within cytoplasm were clearly observed. However, no PEG-Au nanoparticles inside the cell nucleus could be detected and most nanoparticles were agglomerated.

We further examine the dose enhance effect by measuring the proliferation ability under X-ray radiation. The surviving fraction of the cell with or without PEG-Au nanoparticle treatment were almost the same, as can be seen in Fig. 2, indicated that the 500 mM PEG modified Au nanoparticles did not influence the cell viability before X-ray irradiation and therefore confirming their biocompatibility. With X-ray irradiation, Fig. 2 shows clearly the enhancement effect of the PEG-Au, i.e., the increase of X-ray dosages suppress much more the rate of cell proliferation of those treated with PEG-Au. For example, at dosages of 1 or 2 Gy, the survival percentages were 92 % and 58 % or 64 % and 48 % for the control cells and 500 μM PEG modified Au mediated cells. This result clearly indicates that CT26 cells treated with PEG modified Au nanoparticles has lower survival rate, compared to untreated control cells under identical radiation doses.

Understanding the chemical modifications by the radiation and the degree of damage is quite important for potential therapeutical applications. We explored these issues with SR-FTIR, exploiting the exceptional quality of synchrotron radiation to enhance the spatial resolution and signal-to-noise level. Likewise, synchrotron radiation had already expanded the scope of FTIR spectromicroscopy to a variety of biological studies including investigation of cell membranes, proteins and nucleic acids, as well as tissue engineering.

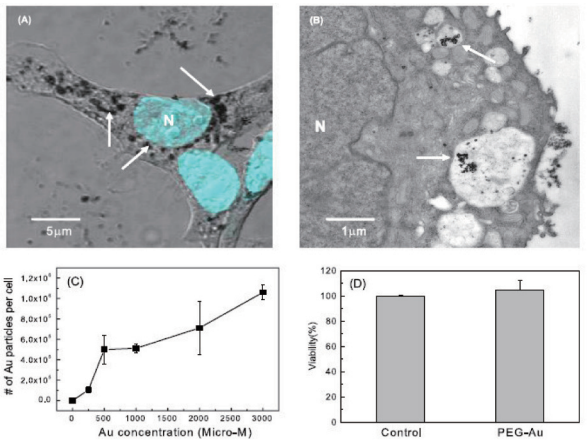

Fig. 1: Results showing that large quantities of pegylated gold nanoparticles in high-density colloidal solutions are internalized within CT26 cells. However, the cell viability of cells is not affected: (A) confocal microscopy image; (B) TEM micrograph; (C) ICP analysis of cell uptake and (D) Alamar Blue assay. CT26 cells co-cultured with pegylated gold nanoparticles for 48 hours. White arrows indicate clusters produced by particle aggregations; N is the nucleus.

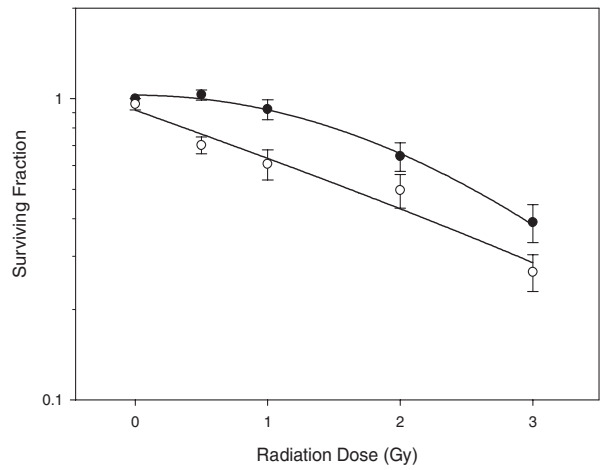

Fig. 2: Radiation survival curves for CT26 cells. The full dots are results for CT26 cells co-cultured for 48 hours, whereas the open circles refer to 48 hours exposure to 500 μM pegylated gold nanoparticle colloid -- before and after X-ray irradiation with the indicated dose). The data represent the mean ± a standard deviation of the results from four independent experiments.

The standard FTIR spectra of CT26 cells exhibit features corresponding to proteins amide A, lipid, proteins Amide I and proteins Amide II at 3300, 2936, 1640 and 1540 cm^{-1}. Previous studies indicated that the shape of the Amide I band can be influenced by the overall secondary structure of cellular proteins including α-helix, β-shift, turn and random coils. We found no difference in this band between the control cells and the cells exposed to the nanoparticle colloid.

As shown in Fig. 3(a), the most striking X-ray-induced spectral variation is the appearance of a new peak at ~1730 cm^{-1} totally absent for non-irradiated specimens. This spectral feature was attributed to the formation of C=O bonds related to the lipid and protein endoperoxides, early state of apoptosis or cell death. The C=O bond peak intensity after X-ray irradiation is higher for cells treated with to pegylated gold nanoparticles than for untreated cells, confirming the nanoparticle enhancement effect.

Gaudenzi *et al*. argued that the additional C=O vibration can be attributed to cytotoxicity. Our SR-FTIR results confirm therefore the cytotoxicity tests: pegylated gold nanoparticles are not toxic before X-ray irradiation. In order to take into account the effects of the individual cell thickness on the C=O peak strength, we derived the intrinsic absorbance by normalizing the peak intensity to that of the protein Amide II band (Fig. 3(b)). This ratio is increased by X-ray irradiation, from 0.05 ± 0.01 to 0.18 ± 0.01 for cells treated with pegylated gold nanoparticles. Considering the radiation survival results (Fig. 2), we can thus use this ratio as a useful indicator to index and evaluate the cell damage.

Our results cannot exclude the possibility of X-ray dose enhancement by intercellular nanoparticles accumulation. However, due to the large amount of nanoparticle uptake produced by our approach, we can conclude that this is a minor effect.◆

Experimental Stations

White X-ray Source
IR Microscopy

Publication

C. J. Liu, C. H. Wang, C. C. Chien, T. Y. Yang, S. T. Chen, W. H. Leng, C. F. Lee, K. H. Lee, Y. Hwu, Y. C. Lee, C. L. Cheng, C. S. Yang, Y. J. Chen, J. H. Je, and G. Margaritondo, Nanotechnology **19**, 295104 (2008).

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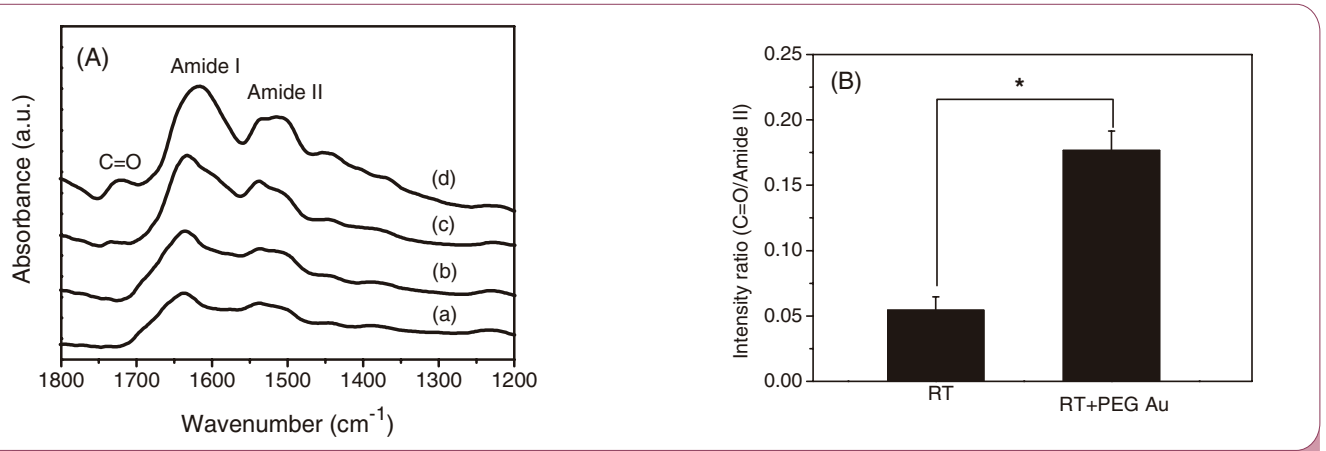

Fig. 3: (A) Synchrotron radiation FTIR spectra of CT26 cells after different treatments. (a) untreated cells; (b) cells after exposure to pegylated gold nanoparticles; (c) untreated CT26 cells after X-ray irradiation; (d) cells exposed to pegylated gold nanoparticles after X-ray irradiation. (B) Intensity ratio of C=O/Amide II for CT26 cells: the results are shown for the same cases, radiation treated (RT) and radiation treated with PEG Au, as in the previous figure. The data represent the mean ± 1 standard deviation of the results from two independent experiments. *, $p < 0.05$, radiation treated versus radiation treated with PEG Au.