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Enhancement of Taxol effectiveness on HeLa cells by narrow bandwidth infrared radiation

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1. Introduction

Infrared(IR) light has been applied in many fields such as night vision, satellite sensing and gas sensor. In biophysical applications, *Escherichia coli* growth and cancer cell proliferation can be regulated by IR exposure [1-2].

As the applications widen, various kinds of IR light sources with narrow bandwidth and reliability are required. Ebbesen discovered extraordinary transmission in 1998 [3]. Extraordinary transmission is due to surface plasmon polaritons propagating through silicon substrate covered with metal perforated by hexagonal gold holes array. Wu et al. demonstrated a high power narrow band tri-layers Ag/SiO_2/Ag waveguide thermal emitter (WTE) and observed that surface plasmon modes are replaced by waveguide modes as dielectric layer becomes thicker than 1μm [4]. Compared to surface plasmon modes, waveguide modes exhibit a narrower bandwidth and the modes do not split at different observation angles.

There are many types of chemotherapy drugs, and Taxol (paclitaxel) is used to treat patients with head, breast, ovarian and lung cancer, which is tightly bound to the microtubule and stabilized it. In other words, Taxol can obstruct microtubule disassembly. As a result, Taxol interferes with the chromosome segregation and spindle assembly during cell division. On the other hand, Taxol blocks the cell in the G2/M phase of the cell cycle as an adjuvant to enhance radiation therapeutic effects [5].

Most previous studies demonstrated radiation therapeutic effect using high energy x-rays with adjuvant Taxol, few low energy IR radiation effect has been studied in conjunction with Taxol treatment. Therefore, in this paper a broad band blackbody emitter and a narrow bandwidth WTE are applied to radiate the cell together with Taxol treatment to study biophysical effects of IR radiation. It is observed that the number and cell sizes of the human cervical carcinoma HeLa cell line treated with Taxol and IR radiation simultaneously are reduced more than those treated independently.

2. Experiments and results

The effect of narrow bandwidth IR radiation in the wavelength range of 3 to 5 μm on the Taxol effectiveness on HeLa cells has been studied. In this experiment, WTE samples and a 3-5μm band pass filter combined with a wide band blackbody source were used for 48hr IR exposure. In order to validate that IR can penetrate through the culture plate, the Perkin Elmer 2000 Fourier-transform infrared spectrometer was used to measure the transmission spectra. Fig. 1(a) shows the transmission spectra of a wide band blackbody source through a plastic 12-well culture plate combined with a 3-5μm band pass filter (red curve).

The wide band blackbody source was fabricated in the following way: A 300 nm thick molybdenum film was deposited on the back side of an n-type silicon substrate as a heating source. With the increasing heating temperature, the silicon substrate increases its emission power. The side view of a wide band blackbody source is shown in Fig. 1(b).

Fig. 1(c) shows the side view of a tri-layer Au/SiO_2/Au WTE on the silicon substrate. The WTE was fabricated in the following way: A 300 nm thick olybdenum film was deposited onto the substrate backside as a heating source. A 200 nm thick gold film was then deposited on the silicon substrate to retard the heat radiation from the substrate. A silicon dioxide dielectric film was condensed onto the gold film by plasma enhanced chemical vapor deposition (PECVD). The thickness of silicon dioxide film was set at 1400 (sample A) and 1600 nm (sample B), respectively. Finally, a second 100 nm thick gold film was deposited onto the dielectric film, and perforated by hexagonally arranged circular hole array photolithographically. The lattice constant and holes diameter of samples were 3.0 and 1.5 μm, respectively. The patterned area of samples were 2 × 2 cm. A Perkin Elmer 2000 Fourier-transform infrared spectrometer was used to measure the thermal emission spectra of WTEs. Fig. 1(d) shows the peak emission wavelengths of the two emitters were 4.2 and 4.8 μm for samples A and B, respectively. The full width at half maximum (FWHM) for each WTE was about 0.5 μm, and the radiation intensity was about 2.5 mW/cm^2 at 120℃.

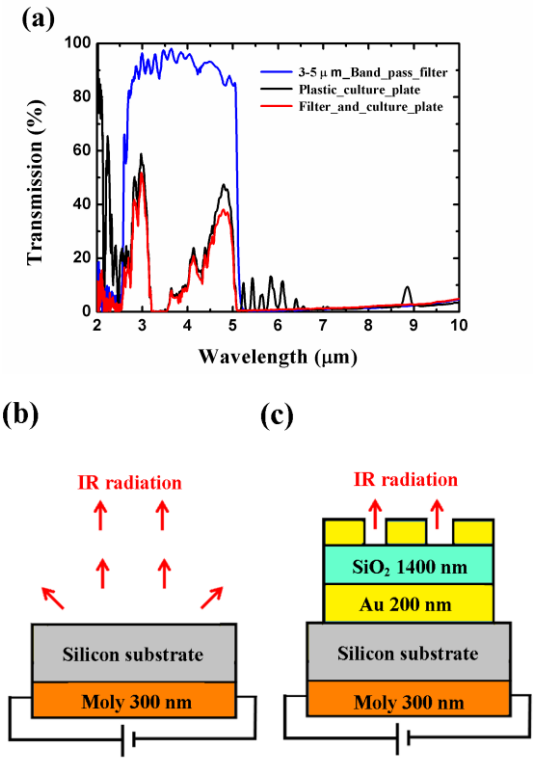

Fig. 1 (a) The transmission spectra of a broad band blackbody source shown in (b) through a plastic 12-well culture plate combined with a 3-5μm band pass filter. The side views of a (b) wide band blackbody source and (c) tri-layers Au/SiO_2 /Au waveguide thermal emitter (WTE).

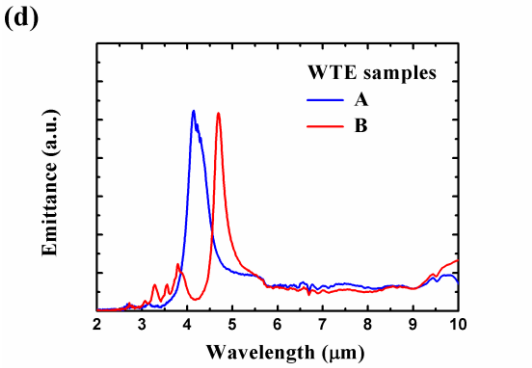

Fig. 1 (d) The peak emission wavelengths of waveguide thermal emitters were 4.2 and 4.8 μm for samples A and B, respectively.

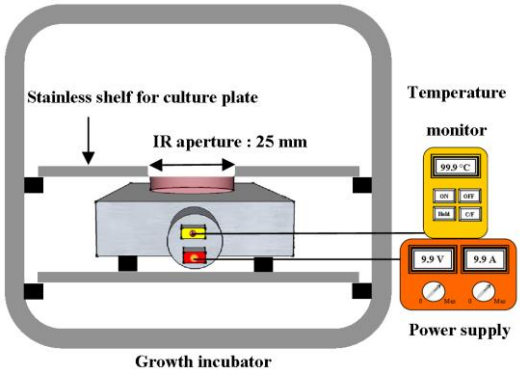

Fig. 2 The facility of an incubator equipped with the narrow bandwidth waveguide thermal emitter.

The arrangement of the apparatus are shown in Fig. 2. A SCI-165DC cell growth incubator (ASTEC Technology Co., Ltd.) was used to maintain temperature at 37 ℃ and 5% CO2. IR from the WTE was passed through a calcium fluoride (CaF_2) window of the metal chamber, and then radiated those attached cells in the well of 12-well culture plate. The cells were incubated in RPMI 1640 medium containing 10% fetal bovine serum and 0.5% penicillin-streptomycin overnight. At the beginning of experiment, the overnight medium was removed and replaced by new medium. Then Taxol(10nM) and IR exposure were added depending on different treatments for 48hr.

At the end of experiment, the cells were measured by Scepter™ cell counter that was able to accurately count by using the Coulter principle to discriminate cell diameter at sub-micron resolution. Scepter™ Software Pro was then used to perform data analysis to determine cell diameters and relative numbers of cells that can be depicted as color dot plots as sown in Fig. 3(a). Because control cells (untreated) consisted of a large number of debris under cell diameter 9μm and nearly no cell existence above cell diameter 19μm, so cell diameter between 9 to 19μm are required for each sample. Fig. 3(b) shows the relative total cell number Sample/Controld. The treatment of Taxol 10 nM without IR exposure was 77%. However, those treatments of Taxol 10 nM with IR wavelengths of 4.2, 4.8, and 3-5 μm were 69, 58 and 64%, respectively.

It is believed that IR irradiated the cell with Taxol treatment can lead to enhanced Taxol effectiveness. This is possibly attributed to the thiol functional group (-SH), because its stretching vibration bands are in the wavelength range of 4 to 5 μm. Thiol groups play an important role in biology such as the functional group of amino acid cysteine.

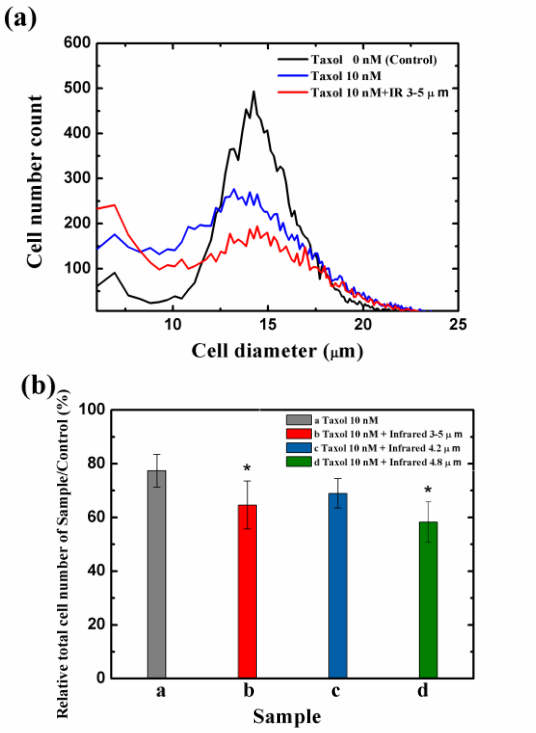

Fig. 3 (a) Cell diameters and relative numbers of cells under different treatments. (b) The peak emission wavelength 4.8 μm enhanced Taxol effectiveness on human cervical carcinoma HeLa cell line for 48hr. *P < 0.05 vs. the cells treated with Taxol 10 nM.

Cysteine oxidation can regulate catalysis reaction, signal transduction, and lead to the formation of disulphide bridge [6]. In summary, IR may enhance the vibration of protein thiol groups and contribute to the Taxol effectiveness on HeLa cells.

3. Conclusions

The enhancement of Taxol effectiveness on human cervical carcinoma HeLa cells by narrow bandwidth IR radiation in the wavelength range of 3 to 5 μm have been studied. It is found that the peak emission wavelength at 4.8 μm and 3~5 μm broad band source can enhance Taxol effectiveness and reduce total cell number significantly. However, the molecular mechanism by which gene expression activated remains unclear, more research is needed to understand the effect of narrow bandwidth IR radiation on the cancer cell with Taxol treatment.

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