Conditions of synthesis for PpIX*

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The characteristics of ALA absorption spectra under different conditions are studied with spectrometers. The results indicate that the wavelength of ALA, corresponding to the strongest absorption peak, is 282 nm under different conditions. The content of PpIX synthesized is in direct proportion to the density of Jurkat cells. The most suitable culture time is 8 hours in order to synthesize more PpIX. FCS has a block effect on the synthesis of PpIX.

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Photodynamic therapy (PDT) is an effective treatment for cancer[1-3]. In PDT treatment, a tumor-localizing photosensitizer is administered, and stimulated by light with special wavelength to initiate a chemical reaction leading to the death of tumor cells. In the course of PDT based on the 5-aminolaevulinic acid (ALA), the photosensitizer stimulated by special wavelength light is Protoporphyrin IX (PpIX). It is safer than HPD as a kind of photosensitizer in ALA-PDT[4]. Because the effect of killing cancers is in direct proportion to the concentration of PpIX[5], it is significant to study the factors affecting the concentration of PpIX coming from ALA after a series of processes inside cells.

However, it is difficult to measure directly the concentration of PpIX, because of the very low concentration of PpIX inside cells. Synthesizing PpIX will expend the content of ALA, which indicates that the concentration of survival of ALA is in inverse proportion to the concentration of PpIX synthesized inside cells on condition that the concentration of ALA added to cells keeps constant[5-8]. Therefore we studied the effect factors of synthesis of PpIX via the effect factors of expenditure of ALA. In fact, the effect of ALA-PDT can be obtained by measuring the concentration of ALA[9-11].

The measurement of the spectrum characteristics of photosensitizer is one of the most efficacious methods for judging the content of the photosensitizer when its concentration is very low[12-15]. Therefore, we investigate the effect factors of the concentration of the survival of ALA with spectrum method, to study the effect factors to synthesize PpIX in this paper.

The materials are as follows: ALA (Sigma Company, America); RPMI-1640 (Gibco Company, America); FCS (Sijiqing Company, China); Jurkat cell (Zhongshan University).

All samples which need to be cultured were cultured in a CO_2-incubator at 37℃, with 80% relative humidity and 5% CO_2 conditions. In order to gain Jurkat cells in the logarithmic phase for the experiment, we detected the growth curve of Jurkat cells. Jurkat cells were cultured in a CO_2-incubator, then the growth curve was obtained and shown in Fig. 1. Obviously, the logarithmic phase of Jurkat cell is between 4th and 6th day during the culture. Therefore, Jurkat cells for the experiment were selected from the period.

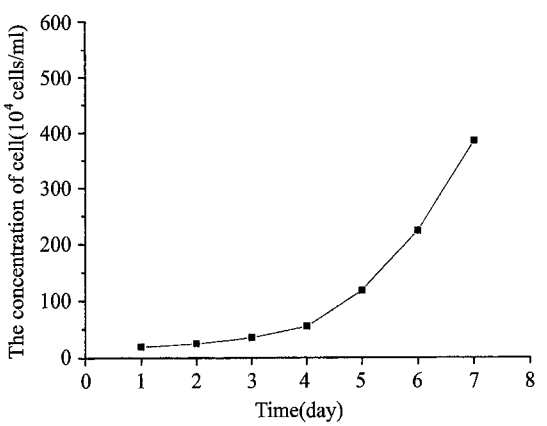

Fig. 1 The growth curve of Jurkat cells

In all experiments in the paper, the Jurkat cells were treated under sterile conditions. The medium and flask were changed weekly to prevent the cells from poisoning in culture flask. All the absorption spectrum data in the paper were obtained by Lambda35 UV/IS spectrometer

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(American Perkin Elmer Company).

The ALA was dissolved in the RPMI-1640 culture medium without FCS (fetal calf serum). The absorption spectrum of pure ALA can be gotten by eliminating the effect of the culture medium. The results were shown in Fig. 2. The wavelength, corresponding to the strongest absorption peak, was 282 nm according to Fig. 2. The strongest absorption peak was considered as the characteristic peak of ALA, and we can judge the content of the survival of ALA by the measurement of the intensity of the characteristic peak of ALA, so as to judge the content of synthesis PpIX.

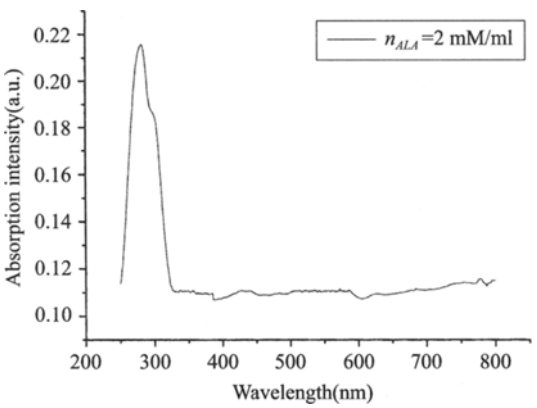

Fig. 2 The absorption spectrum of pure ALA

The different concentration solutions of ALA were mixed with the Jurkat cell solution with density of 5.4 $\times 10^4$ cells/ml, then the mixture solutions were cultured in an incubator for 24 hours. The absorption spectra of ALA solution of different concentrations obtained by eliminating the effect of the Jurkat cell solution were shown in Fig. 3. It can be seen that although mixed with the solution of Jurkat cell, ALA still keeps its character peak of 282nm wavelength. We also know that the absorption intensity is in direct proportion to the concentration of ALA, which indicates that the content of ALA can be judged according to its absorption intensity: the stronger the absorption intensity is, the larger the content of ALA is.

The ALA solution of 2.0 mM/ml was mixed with the Jurkat cells solution of different densities. The absorption spectra of ALA were obtained after the mixture solutions were in incubation for 24 hours. The results are shown in Fig. 4. It can be seen that the larger the Jurkat cell density is, the stronger the corresponding absorption intensity of ALA, which indicates that the content of PpIX synthesized in the higher density of Jurkat cells is larger than that in the lower density of Jurkat cells. Higher density of Jurkat cell is beneficial to the synthe-

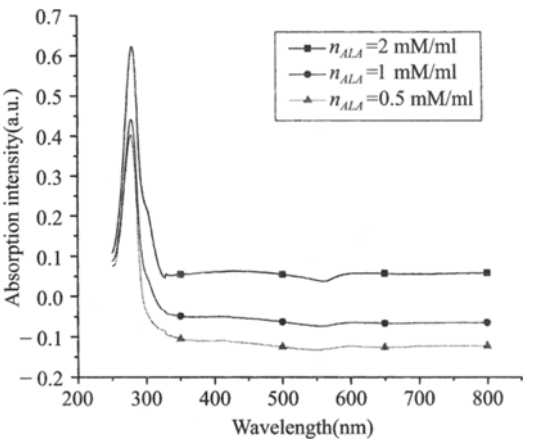

Fig. 3 The absorption spectra of ALA of different concentrations

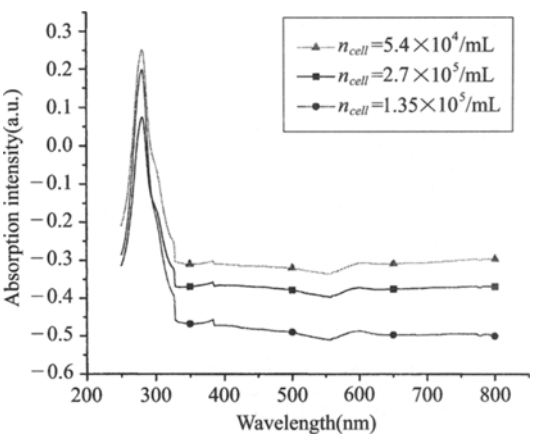

Fig. 4 The absorption spectra of ALA at different cell densities

The ALA solution of 0.7 mM/ml was mixed with the Jurkat cell solution of 2.7 $\times 10^5$ cells/ml and the FCS with different concentrations. The absorption spectra of ALA at different concentrations of the FCS were obtained by eliminating the effect of the background after the mixture solutions were in incubation for 24 hours. The results are shown in Fig. 5. It can be seen that the FCS has a block effect on the synthesis of PpIX. The larger the concentration of the FCS in the mixture solution is, the larger the survival of ALA is.

The sample of cell solution of 5.4 $\times 10^4$ cells/ml mixed with ALA solution of 2 mM/ml was cultured in different time. The absorption spectra of ALA at different culture time are shown in Fig. 6. It can be seen that the concentrations of survival of ALA mixture solutions are non-linearly related to the culture time. The intensity of absorption peak of ALA is the least when the culture time is 8 hours, in the other words, the most suitable

sized.

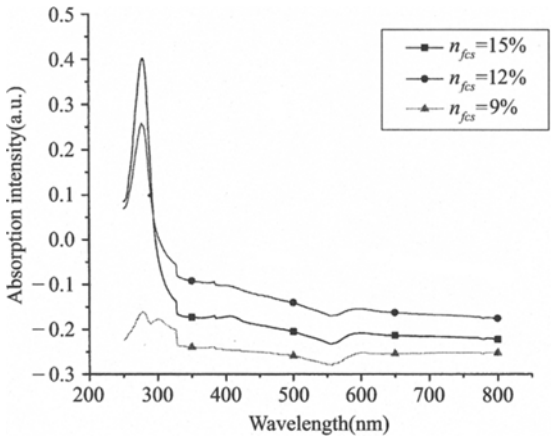

Fig. 5 The absorption spectra of ALA at different concentrations of FCS

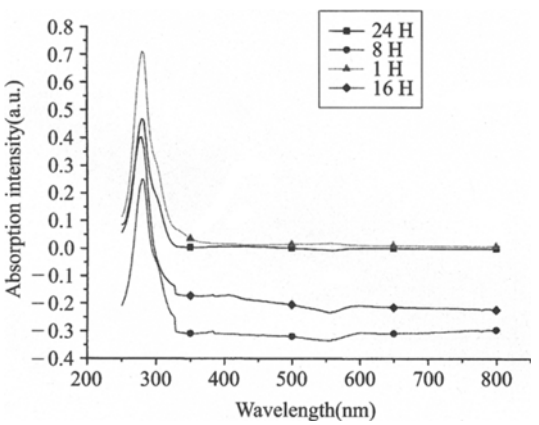

Fig. 6 The absorption spectra of ALA at different culture time

From this research we can draw the conclusions: the wavelength of the strongest absorption peak of ALA is 282 nm under different conditions ; the content of PpIX synthesized is in direct proportion to the density of Jurkat cells; the most suitable culture time is 8 hours in order to synthesis more PpIX; FCS has a block effect on the synthesis of PpIX.

Abbreviations

ALA	5-Aminolevulinic acid
DMSO	Dimetyl sulfoxide
FCS	Fetal calf serum
HPD	Hematoporphyrin derivative
MTT	3-(4, 5-dimethylthiazol-2-yl) 3,5-dimethylthiazo-lium-bromade
OD	Optic density
PpIX	Protoporphyrin IX

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