RAPID DRUG SUSCEPTIBILITY TEST OF MYCOBACTERIA TUBERCULOSIS BY SINGLE CELL TRACKING METHOD IN 3D AGAROSE MATRIX

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ABSTRACT

Tuberculosis (TB) is one of major global health problems. To make matters worse, the drug resistant TB such as multi-drug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) is spreading out throughout the world which means the drug susceptibility test (DST) is necessary to treat the TB patients. To reduce the time for DST, we applied the single cell tracking method. Mycobacterium tuberculosis (MTB) was inoculated in the agarose matrix which is used for 3D culture environment and drug delivery media. For single cell tracking of MTB, we fabricated a microfluidic chip which contains a chamber for agarose matrix. By observing the response of MTB to the drug in single cell resolution using microscopy, we could differentiate the growth and non-growth of MTB according to the TB drug and its concentration and determine drug susceptibility only in five days which is concordance with the DST results from conventional method. This rapid DST method can reduce DST time dramatically and be used for fast and accurate treatments for TB patients leading the increase of the cure rate.

KEYWORDS: Mycobacteria Tuberculosis, drug susceptibility test, single cell analysis, 3D culture

INTRODUCTION

The infectious disease that threatens health of one third of the world’s population is Tuberculosis (TB). Every each year about 1% of the population in the world contract tuberculosis and 1.3 million people were killed by TB in spite of existence of anti-tuberculosis². Consequentially multdrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) is gradually increasing due to poor managing the TB patients or TB suspect². To reduce transmission of TB and improve outcomes for TB patients, a rapid and accurate treatment after drug susceptibility test (DST) is necessary. For rapid DST of MTB, we invented a new chamber to make a thin layer of agarose and MTB mixture and have open surface on top of the thin layer for supplying culture media and drugs. The size of culture medium reservoir is enough for long term incubation. For immobilization of MTB, agarose was used as a cell fixing material and produced a 3D culture niche. The culture medium and drug were diffused into the agarose matrix where single cell of MTB was tracked using time lapse microscopic imaging method. The acquired images were automatically processed to produce quantitative digital information about the MTB growth. From the data, the MIC values of drugs were determined the MIC value only in 5 days.

EXPERIMENT

For microscopic time lapse imaging of MTB, a test chip is needed to provide the immobilization of MTB in agarose for a sufficient delivery of drugs and optical transparency. To satisfy these requirements, a new chip called a disc agarose channel (DAC) chip was designed and fabricated. (Fig. 1a) The DAC chip is composed of a disc-shape channel where the agarose and MTB mixture is loaded. The structures of open space and hole was used for enhancing the diffusion of the culture medium and drugs (Fig 1b). The depth of the disc shape channel is determined by the height of a spacer and its value was 300 μm. For DST of MTB, a long time of incubation is necessary and the culture medium should be supplied sufficiently to MTB. Therefore, each well is designed in the size of a single well in 24 well plate. In
terms of dimensions, the length and height of the well is are 11mm and 10mm, respectively, and it contains about 1mL of medium which is suitable of for more than 1 month of incubation. The DST process is described in Fig. 1d. The single cell tracking method with time lapse method was used for measurement of the MTB growth under various drug conditions. The MTB cell in the DAC chip were tracked every day (Fig. 1e).

RESULTS AND DISCUSSION

For the uniform supply of culture media and drug, the boundary area between agarose and liquid medium of agarose mixture in DAC chip is needed (Fig. 2a) For visualization of diffusion characteristics of agarose, rhodamine B (molecular weight: 479.02 g/mol) which have the similar molecular weight with the TB drugs was used. At 1 μg/ml of concentrations, the fluorescent signal became uniform only 30 min after loading of fluorescent dye (fig. 2b). The microscopic time lapse images with CCD camera were processed to digitalize the MTB growth under TB drug conditions. The raw images from CCD camera were processed to a binary format in Fig. 3a. Using the sequential digital date, the growth curve according to the time was plotted in Fig. 3b. By setting the threshold value, we determined drug susceptibility of MTB strains under various concentration of TB drugs. To validate the DAC system for rapid DST, virulent standard strain of MTB, H37Rv and clinical strains of MDR and XDR strains were tested to determine the susceptibility of isoniazid, rifampicin, streptomycin, and ethambutol. It was used for quality control and as a comparison target that direct phenotypic LJ culture tests are regarded as the ‘gold standard’ of DST. The MIC values of isoniazid, rifampicin, streptomycin, and ethambutol against H37Rv, MDR, and XDR that were determined by using the DAC system which is concordance with the DST results from conventional method are shown in table 1

CONCLUSION

In summary, the single cell tracking of MB in the agarose matrix produced a rapid DST in 5 days. The agarose matrix provided 3D culture environment which is suitable for MTB culture and DST by supplying culture media and drugs. The standard strain, H37Rv, MDR and XDR TB strains were tested in this platform and resulted in comparable data with the conventional method. This DST methods could be used as a rapid DST method and help contribute to reduction of the global health problems related in TB.

Figure 1. A) Optical micrograph of 3D culture chamber array chip. B) Plane figure of 3D culture chamber. C) Image of MTB incubated 5 days in the chamber. D) Experimental procedure. D1) Empty chamber D2) Loading MTB mixed with agarose D3) Loading of drug in growth media D4) Diffusion of drug and time lapse imaging.
Figure 2. A) Optical micrograph of the boundary area between agarose and liquid medium of agarose mixture molded which is visualized by food dye b) Diffusion of rhodamine B in DAC chip. Image was taken immediately after rhodamine B was loaded. Images ii, iii and iv were taken 10 min, 30 min and 1 hour in sequence. The exposure time was 0.1s. The scale bars represent 1mm.

Figure 3. A) Time lapse images and processed images. 1) and 1)’ Resistant cases. 2) and 2)’ Susceptible cases. Quantification of growth dynamics of resistant and susceptible cases.

Table 1. The result of DST from DAC. H37Rv, MDR and XDR TB strains were tested with 4 primary TB drugs, isoniazid (INH), rifampicin (RFP), streptomycin (SM), and ethambutol (EMB).

<table>
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<tr>
<th>Sample#</th>
<th>H37Rv</th>
<th>MDR</th>
<th>XDR</th>
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<tbody>
<tr>
<td>INH</td>
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<td>&gt; 0.2</td>
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<tr>
<td>RFP</td>
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<tr>
<td>SM</td>
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<tr>
<td>EMB</td>
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<td>&gt; 10.0</td>
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REFERENCES

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