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Prevention and detection of microbial contamination of cancer cells cultured in vitro based on reduced pressure concentration

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Abstract

Prevention of microbial contamination in cell culture refers to the phenomenon that microorganisms such as bacteria, mold or virus intrude into the cell culture during cell culture, causing harm to the survival of cells. The contaminated cancer cells cultured in vitro were selected as the research materials, the 16s rRNA gene sequence was selected as the universal primer for bacterial microorganisms, and the bacterial genomic DNA was extracted by vacuum concentration method. The procedure of PCR for detecting the contamination of cultured cells was designed, and the detection results were analyzed. In vitro culture of cancer cells contaminated with Staphylococcus albus, Escherichia coli and Pseudomonas aeruginosa, fragments consistent with the target fragments can be amplified from the culture supernatant. The results showed that the reduced pressure concentration method reduced the difficulty of detecting bacterial microbial contamination in cancer cells cultured in vitro, and could find the contamination early and buy time for saving precious cells. It has the advantages of low cost and high efficiency, and has high popularization and application value.

Keywords

Concentrate under reduced pressure; In vitro culture; Cancer cells; Microbial contamination; Prevention; Examine.

1. Introduction

The pollution has always plagued scientific research and clinical medical workers, which may lead to wrong experimental results and even scientific research failure, and may lead to wrong test results, delay patients' treatment or treatment failure. There are many factors causing pollution in medical cell laboratory, such as aerosol generated during experimental operation, air with powder and microorganism entering sterile laboratory, polluting reagents, poor aseptic concept of operators and technicians, and nonstandard aseptic operation, etc. Therefore, it is very important to detect and eliminate bacterial microbial contamination in cancer cell culture in vitro. It has been reported that PCR technology is used to detect mycoplasma contamination in culture [1]. However, due to the DNA extraction of culture and the high experimental conditions and cost required by PCR technology itself, it is not easy to popularize in general laboratories. If the designed primer can not cover all mycoplasma species, it will easily lead to false negative of the test specimen [2].

In this project, special genes are searched from microbial resources, and functional proteins are obtained through gene cloning and expression, or genes from different sources are assembled in microorganisms by means of synthetic biology and metabolic engineering, and a microbial cell factory is designed and constructed artificially, and microorganisms are used as cell factories to produce useful substances. Through this project, students can learn the basic knowledge and technology of molecular biology and synthetic biology and metabolic engineering.

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In this paper, DNA was extracted by vacuum concentration method, universal primers of 16S rRNA specific sequences of bacteria were synthesized, and the culture was detected by PCR amplification method, which was really cheap, sensitive, simple and fast. Moreover, the contamination of bacteria and microorganisms in cancer cells cultured in vitro can be detected at an early stage, which is also of great significance for the rescue after contamination.

2. Types of microbial contamination in cancer cells cultured in vitro

2.1 Impurity cell pollution

Primary cells are digested by tissue blocks. In the process of digestion, because the cut tissue blocks are not pure enough and the digestion time is not well grasped, it is easy to digest and mix impurity cells into primary cancer cells cultured in vitro. Impurity contamination can be judged by microscopic observation of cell morphology. If impurity cells are mixed in, the cell population can be purified by re-digestion, so as to achieve the purpose of purifying cells.

2.2 Mould contamination

Mold pollution often occurs in wet seasons or wet operating environments. Mould contamination can be detected by observing cell growth and culture medium with naked eyes. Mould will not cause turbidity of nutrient solution in a short time, but obvious mould colonies can be seen in the cell bottle wall.

The mycelia of mold can be seen under microscope, and the color of nutrient solution often turns yellow. Mould contamination can deform and exfoliate cells. The fungus has a strong tolerance to the environment, and it is difficult to remove it by ordinary disinfectant, so it brings serious harm to the operating environment.

2.3 Bacteria contamination

Bacterial contamination is common in cell culture. It is mainly caused by improper operation, lax disinfection of equipment, or undetected bacteria with hidden contamination of various nutrients. Even if antibiotics are added into the cell culture solution, the bacterial reproduction is in a state of inhibition, and the cell growth is not obviously affected and is not easy to be observed by naked eyes. However, with the failure of antibiotics, the bacteria in the nutrient solution proliferated and caused pollution [3].

2.4 Mycoplasma contamination

Mycoplasma can form a symbiotic relationship with host cells, which makes the pollution expand continuously. Once mycoplasma contamination occurs, mycoplasma can be deposited on the surface of the object through potentially infectious droplets and adsorbed dust generated during liquid transfer and dumping. This kind of polluted dust can survive for days or even weeks, which pollutes the operating environment. If it is not treated in time, it will also cause cross-contamination or re-contamination.

2.5 Viral pollution

Virus pollution is the most hidden, and once polluted, it is difficult to detect by conventional methods. The virus particles are small and invade the cells, which is difficult to be observed by ordinary optical microscope. The cell culture medium of virus-contaminated cells is clear and unchanged. Some pathogenic viruses can cause cytopathic phenomena such as cell rounding, swelling and shedding. According to this phenomenon, whether cells are infected with foreign viruses can be judged.

3. Materials and methods

3.1 Take sample

In view of the three times of serious pollution, air from different directions in sterile culture room and air from all levels of incubator were collected, cotton swabs were used to purify the countertop, and

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five air points and suspicious cell culture media were collected at the same time at the four top corners and the center of the countertop.

3.2 Culture and detection methods

And the culture method is as follows:

(1) Scrape the above bacterial ice crystals respectively, inoculate them into LB culture medium, and cultivate them overnight at 37℃, and inoculate 50μl of the above cultures into the newly passed cancer cells in vitro;

(2) Collect culture supernatant of each group, centrifuge, discard supernatant, add 200μl PBS to make resuspension, boil in boiling water for 5min, repeat centrifugation, suck out supernatant, add 200μl isopropanol precooled by ice, stand at -20℃ for 15min, repeat centrifugation, discard supernatant, and resuspend with 50μl TE buffer;

(3) PCR was carried out with universal primers for bacterial 16S rRNA specific sequences. Upstream primer (A-17):5' -GTT TGA TCC TGG CTC AG-3'; Downstream primer (3-17):5' -AAGGAG GTA ATC CAG CC-3'; The amplified fragment is about 1500bp.

4. Result

The experimental results show that:

(1)The culture supernatants of cancer cells contaminated with staphylococcus albus, Escherichia coli and Pseudomonas aeruginosa in vitro all amplified fragments consistent with the target fragments, with a size of about 1500bp;

(2)The concentration of bacterial genomic DNA prepared by vacuum concentration method was 13 μ g ml, and after 10 times dilution, 1μl was taken for PCR amplification. When the concentration was reduced to 53fg.ml, the amplified band was still visible at 1500bp.

See Table 1 for the observation of the results of the three cultures before intervention.

Table 1 Results of pre-intervention culture

Sample collection characteristics	Incubation time	
	24h	48h
Air in different orientations of culture room	Clones of 2~4mm in size can be seen, which are gray and round, with neat and opaque edges	4CFU / φ10cm·15min,White has feet
Gas at all levels of incubator.	Clones of 2~4mm in size can be seen, which are gray and round, with neat and opaque edges	3CFU / φ10cm·15min,White has feet
Clean 5 air points of workbench	Undeveloped bacteria	It can be seen that the middle point clones grow with white feet
Doubt cell culture medium	Slightly turbid, with a white film visible on the liquid surface	Visible white film, white enough
Cotton test sample of culture medium	Clones with gray, round, neat and opaque edges and 2~4mm in size can be seen	Visible clones, white feet

In the culture medium before intervention, a large number of bud spores connected into long chains and a large number of hyphae can be seen. In addition, spherical bacteria were found in some cultures, which were gram-negative cocci and gram-positive cocci by gram staining, and were identified as Acinetobacter baumannii and Candida albicans by Vitek2-Compact automatic microbiological analyzer. No clonal growth in culture medium was observed after intervention.

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5. Discussion

Once the cultured cells are contaminated by microorganisms, they can only be discarded, which will greatly affect the subsequent research work. Pathogens of microbial contamination in cell culture medium include fungi, viruses and bacteria. Contaminated cell culture solution turns yellow, and obvious blocky or flaky colonies can be seen on the bottle wall, so it can be found without special detection methods [4-5]; However, it is often necessary to establish a reference system according to the specific types of viruses, and choose independent detection methods for detection. Ordinary laboratories are not easy to have corresponding equipment conditions, and virus pollution is rare compared with the previous two pathogen pollution, so it is mainly prevented by improving equipment disinfection and aseptic operation conditions [6].

It is a common problem in basic medical research that cell culture solution is contaminated by bacteria, microorganisms and mycoplasma. Once the cell culture medium is contaminated by bacteria or mycoplasma, its morphology and products are greatly different from those of normal cells cultured in vitro, and will affect the subculture cells. Although some cultured cells contaminated by bacteria can save their experimental value through the application of antibiotics, for antibiotic sensitive experiments or prospective experiments, the use of antibiotics to save contaminated cultured cells may not be worth promoting. In previous studies, PCR technology was often used to detect bacterial contamination in cultured cells. However, DNA extraction and PCR detection technology of bacterial microorganisms required higher costs and experimental conditions, and it was difficult for ordinary laboratories to meet the corresponding funding and equipment conditions [7-8].

In this experiment, the bacterial genomic DNA was extracted by vacuum concentration method, which was low in cost, simple and easy to operate. When the template amount was reduced to 53fg/ml, the target band could still be amplified by PCR, which indicated that this method was sensitive and rapid, and could quickly detect the existence of pollution at an early stage, which was very important for saving some precious cells. In addition, the 16S rRNA gene sequence is a unique gene sequence of prokaryotes and is relatively conservative, which is regarded as the molecular evolution clock in the classification of bacteria [9]. In this experiment, this segment was amplified by universal primers, and the species of pollutants could be further confirmed by sequencing, so as to carry out targeted rescue.

6. Prevention of microbial contamination of cancer cells cultured in vitro

Because cells have no ability to resist microorganisms in vitro, once the cells are contaminated, most of them are difficult to remove. If it is not an important cell, it is generally recommended to discard or eliminate it. The key to cell culture is to prevent pollution, not to remove it. Prevention is the best way to prevent pollution during cell culture. When the cells are polluted early or slightly, if they can be treated in time, some cells can be saved.

The use of antibiotics to prevent pollution is usually better than that of a single antibiotic, and some antibiotics only have inhibitory effect but not bactericidal effect. If the dosage is too large, the cells can't tolerate it and have toxic effects on cells or cell lines. Therefore, special attention should be paid to the characteristics of different cell cultures and the differences in antibiotic tolerance. To select antibiotics for specific cells, firstly, the types and doses of drugs are screened to select antibiotics that can inhibit polluting microorganisms and do not affect cell growth.

The key to pollution treatment is to find out the source and way of pollution. When judging the source and way of cell pollution, we can judge by pollution probability. When raw materials such as nutrient solution are polluted, the whole batch of cells are polluted. If the pollution is caused by misoperation, only a small number of cells are polluted.

It is the main way of airborne microorganism transmission. If the place where the cell culture is operated is not tightly isolated from the outside or the disinfection is insufficient, the unclean air from the outside will enter the culture system and cause pollution. The culture environment cannot be

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located in a ventilated place. Sterile operation should be carried out in the ultra-clean table. Wear a sterilized mask when working, so as to avoid external pollutants entering the operation surface and causing pollution due to conversation and cough. If a large number of cells need to be cultured, the environmental cleanliness should reach 10,000. Regularly check the bacterial content in the air of the ultra-clean platform and the culture site. Sterile workbench and filter screen should be cleaned regularly, wiped clean with 75% alcohol after cleaning, and irradiated with ultraviolet rays for more than 40 min. Irradiating various culture plates for more than 3 hours can clean the air in the environment to the maximum extent.

All kinds of culture vessels and instruments in direct contact with cells will directly affect the growth of cells and lead to the pollution of nutrient solution if they are not thoroughly disinfected and washed. Before culturing cells, the instruments to be used should be classified and thoroughly disinfected. For example, for disinfection of glass instruments and glass culture bottles, high-pressure steam sterilization for more than 30min or dry baking sterilization at 140℃ for more than 2h should be used to completely eliminate all kinds of microorganisms adsorbed on the instruments.

7. Conclusion

In this study, the method of vacuum concentration was selected for crude extraction of bacterial genomic DNA, which was low in cost and had no special requirements for operators. This study confirmed that the extraction of bacterial genomic DNA by vacuum concentration method reduced the difficulty of rapid detection of bacterial microbial contamination in cancer cells cultured in vitro, avoided the influence of contaminated cells on experimental results, and bought time for the rescue of precious cells. At the same time, it has the advantages of low cost and high efficiency, and can be further popularized and applied in basic hospital laboratories.

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