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Microbiological contamination in stem cell cultures

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Abstract

Cell therapy and regenerative medicine are potentially two of the most exciting aspects of the novel therapeutic methods currently under development. However, these treatments present a number of important biosafety issues, like the possible transmission of microorganisms to the recipients. The most common potential form of contamination in these cell products is by bacteria (including *Mycoplasma*), yeast and fungi. In our study, 32 stem cell lines and feeder cell lines were analysed. There were 19 contaminated cell passages (12%). The main contaminants were gram positive cocci and *Mycoplasma* species, followed by gram negative rods and gram positive rods. The *Mycoplasma* contamination rate was 4%. Stem cell banks and other research centres aim to screen all processed stem cell lines for these microorganisms, and to assure that no contaminants are introduced in the banking procedures. It is a standard part of current good practice in stem cell banks to carry out routine microbiological controls of the stem cell lines and to work in a controlled environment to reduce the probability of contamination in the final product.

Keywords: Stem cell cultures; Microbiological control; Cell therapy; Good practices; Clean rooms

1. Introduction

Clinical treatment with stem cells consists of the transplant of these cell products to patients by means of systemic infusion or local injection, which raise unprecedented opportunities for the treatment of many diseases and traumas.

In Spain, Andalusia was the first region to pass a law (Law 7/2003, on October 20th 2003) about the research regulation using human embryos, non-viable for *in vitro* fertilization techniques and which have been stored in liquid nitrogen for more than 5 years. Later, a Spanish Royal Decree (2132/2004), with the establishment of the requirements and procedures to seek the development of research projects with stem cells obtained using spare embryos, was published on October 29th 2004. Recently, the Law 14/2006, concerning assisted reproduction techniques, which deals with the legal order on health care and research, above all in regenerative medicine (e.g. stem cell research, somatic cell nuclear transfer) was passed on May 26th 2006.

After the regulation of the research activities in our country, scientists can legally handle stem cell lines and carry out clinical trials with these cells. For these purposes, stem cell banks and other research centres must assure the quality and safety of these cells, and these objectives are particularly important in avoiding the transmission of infectious diseases to the recipients of these cell products.

Quality assurance is important in all aspects of cell culture. Good practice in the laboratory and the frequent monitoring of the stem cell lines is vital for any research centre or cell bank that handles cell cultures, and should be mandatory if these cultures are intended for clinical use in cell therapy and regenerative medicine. Also, this fact is important in order to avoid the transmission of infections to those who do research with stem cell lines.

In this sense, stem cell banks aim to screen all processed cell lines for serious human or animal pathogens and to insure

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that no contaminants are introduced in the banking procedures. Any microbe contaminating the donated material or being introduced as an adventitious agent during the manufacturing process can potentially multiply during the processing and can also potentially present a serious hazard to the recipients.

The most common potential forms of contamination in cell cultures are bacteria (including *Mycoplasma*), yeasts and fungi, and these can be readily assessed on a routine basis (Cobo et al., 2005). The sources of microbial culture contamination are different: cell contamination from origin, glassware or apparatus (including storage bottles and pipettes), culture media, people and airborne contamination.

To avoid contamination, a formal microbiological control program should be established and introduced to diagnose the main contaminants in stem cell cultures in order to avoid the transmission to possible recipients. In our bank, we have three years of experience with such a control program. In January 2004, we introduced a microbiological control program of both stem cell lines and feeder cells. Briefly, this program consists of the systematic study of bacteria (including *Mycoplasma*), fungi and yeast contamination in all cell lines obtained in our centre in GMP conditions (European Union, 2003). The cells were cultured in different culture media, both solid and fluid (e.g. Trypticase soy agar, fluid thioglycollate, PPLO agar and fluid) in accordance with the Standard Protocols given by the European Pharmacopeia (European Pharmacopeia, 2004a,b). The results of the sterility tests were recorded in registered documents and saved in electronic format in a data base. After this period, we evaluated the data obtained and we present these results below.

2. Materials and methods

2.1. Cell lines and their origins

Cell lines from different origins were used for this study from 1st February 2004 to 30th May 2006. The data records in the aforementioned period, were reviewed retrospectively. Thirty-two cell lines were tested in this study. The origins were diverse: feeder human fibroblasts (2), feeder mouse fibroblasts (12), mouse embryonic stem cells (9), mobilized human bone marrow (2), human bone marrow (5) and human embryonic stem cells (2). The CCD-1112SK cell line is a human skin fibroblast cell line with ATCC accession number CRL-2429. This cell line came directly from ATCC to our laboratory in March 2005 (cell passage 4). The H-181 and H-293 are two human embryonic stem cell lines that were obtained from the Karolinska Institute (Stockholm, Sweden). These human embryonic stem cell lines came directly to our bank in November 2004, at cell passage 46 and 47 respectively. The rest of cell lines were derived from our own bank. We evaluated both human and mouse fibroblasts because these kinds of cells are usually used as feeders in the growth of stem cell lines, so the contaminated feeders could transmit microorganisms to the recipients. The characteristics of these stem cell lines are provided in Tables 1 and 2.

2.2. Good manufacturing practices in stem cell cultures

Our bank is the central node of the bank network of the Spanish Cell Line National Bank. This is an establishment where both clinical use and research is being carried out. For clinical purposes, specific areas called clean rooms are used; however, the research is being carried out in separate conventional laboratories. In our bank, there are two clean rooms, of approximately 45 m^2 of surface each, which are classified as grade B (European Union, 2003). Each stem cell line or feeder cell was handled in a biological safety cabinet type IIA (Richmond and McKinney, 1995) and two cell lines were never handled at the same time in order to avoid cross-contamination.

Moreover, the technicians who handled the cell products always wore adequate clothes consisting of disposable sterile suits, boots, gloves and complete respiratory enclosure. The technicians who were working with these cultures were certified by the Spanish National System of Certification in laboratory techniques, and were all skilled technicians in this field of the biology. Moreover, they attended external training courses periodically and at the same time, an internal training program was run.

Furthermore, in our centre we introduced an environmental monitoring program to operate within a well documented quality assurance system for the air particle control, microbiological air contamination and surface contamination (Cobo et al., 2006).

2.3. Samples

Samples were taken systematically for sterility tests whenever cell lines were received or obtained and upon cell line sub-culture. The tests were carried out in all the consecutive cultures. At least 5–10 ml of specimens were used for sterility tests; after trypsinisation, both supernatant and cells were harvested for microbiological testing. All the cell lines analysed were cultured in antimicrobial free media before sampling for microbiological testing.

2.4. Sterility tests

The detection of bacterial, fungal and yeast contamination was carried out by means of standard culture-based sterility testing, in accordance with the pharmacopeia standards (Pharmaceutical Inspection Co-operation Scheme, 2002; European Pharmacopeia, 2004a,b; US Food and Drugs Administration, 2006). Specimens were cultured on four different media groups which include blood agar (Oxoid Ltd, Hampshire, UK) at 35 °C, both trypticase soy agar and broth (BioMérieux, Marcy-L'Etoile, France) at 35 °C, broth thioglycolate medium (BioMérieux, Marcy-L'Etoile, France) at 35 °C and dextrose Sabouraud agar (Oxoid Ltd, Hampshire, UK) at 30 °C and 35 °C. The incubation conditions are shown in Table 3. Both solid and fluid media were checked daily to ensure early detection of contamination and to enable appropriate actions to be taken as soon as the first signs of contamination became apparent. The fluid media were observed daily for turbidity. Turbid cultures were Gram stained. The broth was subcultured to select agar plates on the basis of the microbial morphology observed in the Gram stain. Later, the microorganisms were identified (see below).

Although some studies were performed to demonstrate the sensitivity and rapidity of automated microbial detection systems (Kielpinski et al., 2005), we used the standard culture-based sterility testing because, at the moment, this is the routine method used in our laboratory and these methods are within the pharmacopeia standards.

On the other hand, *Mycoplasma* detection was carried out by means of both standard culture and polymerase chain reaction (PCR). With respect to standard culture, we used both PPLO agar and broth (Biocult, Madrid, Spain) at 37 °C + 10% CO_2 (see Table 3). *Mycoplasma* detection by PCR method was carried out by the use of the VenorGeM® kit (Minerva Biolabs, Berlin). This kit uses the PCR technology for the detection of *Mycoplasma* and *Acholeplasma* contamination in cell cultures. The primer set is specific to the highly conserved 16S rRNA coding region in the *Mycoplasma* genome. This allows for detection of *M. orale*, *M. hyorhinis*, *M. arginini*, *M. fermentans*, *M. salivarium*, *M. hominis*, usually encountered as contaminants in cell cultures, but also *M. pneumoniae*, *Acholeplasma laidlawii*, *M. synoviae* and *Ureaplasma* species.

2.5. Microbial identification

The microorganisms isolated during the routine microbiological monitoring, except *Mycoplasma* species, were initially characterised by their cell morphology and Gram stain. Later, these microorganisms were identified by means of both an automated system (VITEK 2 compact, Vitek Systems, St. Louis, MO) and a manual system (in several cases in which the automatic