**New Protocol for Microbiological Analysis and bacterial Decontamination of Human Fetal Tissue used for Transplantation for Neurodegenerative Diseases**

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**Abstract**

Transplantation of fetal neural tissue has become a promising treatment and disease management for individuals experiencing various neurodegenerative diseases. Contamination of human fetal tissue by bacteria presents a potential risk of causing infections in the recipient patient’s brain. Therefore, an efficient and applicable microbial decontamination method needs to be developed and required prior to the approval of neurorestorative cell therapy as an effective treatment. A protocol that consisted of subsequent washing steps at various stage of the tissue processing was performed on rat embryonic tissue incubated with high concentrations of microbes, including fungal and bacterial species, to assess the effectiveness of the experimental microbial decontamination. The spectrum of microbial contamination and effect of washing steps on aborted human fetal tissue were analyzed. Of the samples taken during the human fetal tissue processing, 47.7% tested positive for microbial contamination, but subsequently after the washing steps the samples exhibited no growth of bacteria. The data suggested that human fetal tissue for neural repair can transfer microbes of different species to the receiving patient, suggesting the need for decontamination procedures. This report details the importance of decontamination of human fetal tissue in neural repair to reduce transmission of microbes to the brain of the recipient. Thus, highlighting the need for such decontamination processes to further the practice of using human fetal tissue for neurorestorative cell therapy.

**Introduction**

Restorative cell therapy models in neurodegenerative diseases are focused at replacing lost neurons. Although developments in research on pluripotent stem cells have been made, fetal tissue from elective abortions is still considered as the only safe source of cells. Several clinical trials on the transportation of fetal tissue in Parkinson’s disease and Huntington’s disease patients have been performed. These clinical trials have shown that fetal tissue cells isolated during the first trimester have the capability to endure transplantation, acquire a mature neuronal phenotype, and provide a functional benefit (Döbrössy, et al., 2010). Fetal human tissue is derived from elective or medical abortions, therefore a potential infection of the human fetal tissue with microbes from the female vaginal tract could infect the host brain after transplantation. The broad range of colonizing microbes and organisms in the female genital tract have some type of physiological protective and immune modulatory function to the host (Witkin et al). Published data detailing methods for microbiological analysis and risk for contamination of human fetal tissue are very seldom available. Few previous trials have explored strategies to remove contaminations through the use of antibiotic treatment of fetal tissue prior to implantation. Although, it is undetermined whether the time period of antibiotic treatment prior to transplantation is adequate for significant microbial diminution. The effectiveness of antibiotics may be reduced when tissues are contained in tissue storage cases due to the low storage temperatures.

**Recent Progress**

In the study, a protocol was developed based on successive washing steps in order to decontaminate the human fetal tissue. The method was centered on a reputable industrial system known as the BacT/ALERT, which allowed for semiautomated and extremely sensitive detection of microbes (Jimenez, Rana, Amalraj, Walker, & Travers, 2012). The model generated simulated the clinical protocol by artificially contaminating sterile fetal rat tissue with clinically relevant microbes at exceptionally elevated doses (Piroth, et al., 2014). A standardized washing method for the reduction of microbes in the contaminated fetal tissues was designed and applied. Each washing step consisted of five subsequent washing steps with 5 mL each of sterile Hank’s Balanced Salt Solution (HBSS) (Piroth et al., 2014). Contamination was strongly reduced between sample A, the transportation step, and sample B, taken after the first washing procedure. Only a minor reduction of microbes was seen in sample C, after a twenty-four hour storage and second washing procedure. The log rank statistical test was performed to compare the survival distributions of the groups of samples (Bland & Altman, 2004). *Corynebacterium amycolatum* bacterial growth was reduced more than three mathematical log ranks from sample A (105 CFU/ml) to sample B (13.3 CFU/ml); *Lactobacillus jensenii* was reduced by four log ranks from sample A to samples B and C; *Bacillus subtilis* bacterial growth was reduced by six log ranks from sample A to B; *Clostridium sprogenes* was reduced to no bacterial growth in sample B from sample A; *Candida albicans* shows no growth in sample C (Piroth et al., 2014). The microbial contamination was greatly reduced by at least two log ranks for all of the microbes. Recent progress has shown that this protocol has been adapted and used by many scientists in order to reduce the amount of contamination in human fetal tissue and reduce the spread to the host patient. Before this protocol was implemented there was a high rate of contamination with bacteria or fungi in human fetal tissue transplantation (Rice, Hedrick, Flake, Donegan, & Harrison, 1993). The protocol has been adapted for use in studies on the use of stem cell for autism treatment. Recent studies have been performed on neurotransplantation for Huntington’s and Parkinson’s disease and have modified the protocol by developing an inclusion and exclusion criteria for fetal neuronal cells transplantation based on the amount of bacteria, fungi, and viruses present in the donor’s blood. This paper concluded that the decontamination described in the current transcript, in addition to the criteria and distribution of general antibiotics, was effective and allowed for a safe preparation of tissue for transplantation (Lopez et al., 2013).

**Discussion**

The system presented in this report was intended to overcome previous methods’ limitations by using BacT/ALERT bottles to allow for an extremely sensitive detection of microbes. This protocol examined a substantial portion of transportation fluid as well as the washing fluid from subsequent washing procedures to detect the levels of microbial contamination at each step. The study revealed the broad spectrum of probable microbe strains found in first trimester abortions, all of which could possibly infect the brain of the host, were significantly decreased after the subsequent washing steps. In the part of the study regarding microbial decontamination of rodent fetal tissue, they found that there was a significant decrease in microbes after the subsequent washing steps but not complete eradication in the final sample. In the microbial decontamination of human fetal tissue, they found that the samples B and C did not contain any contamination after the subsequent washing steps (Piroth et al., 2014). The results were valid, and the researchers provided explanations for the results they incurred in the study. The success and safety of fetal cell transplantation was dependent upon various microbiological aspects. A protocol was designed and implemented for the testing and decontamination of microbes in fetal tissue. The subsequent washing outlined in the protocol was adequate in the removal of microbes from human fetal tissue samples. Contamination after washing steps should be carefully monitored and pre and postoperative antibiotics should be administered to reduce the risk of infection in fetal neural transplantation. The results indicated that subsequent washing steps should be performed to greatly reduce the microbial contamination of the fetal tissue into the host brain and antibiotics should be administered as well. This should reduce the overall likelihood of infection in the host brain and increase the success rate of fetal neural transplantation. The study addressed that it was still unclear if infection in the host brain was due to potential contamination of the fetal tissue during the surgical procedure, the abortion, or microbial contamination during tissue processing.

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