

THE PRESENCE OF P22 BACTERIOPHAGE IN ELECTROCAUTERY AEROSOLS

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ABSTRACT

Electrosurgery units, including lasers and electrosurgical cauterizers, are commonly used for both inpatient and outpatient procedures. The generation of an aerosol plume, associated with the use of these devices, is a result of many factors including: 1) direct aspiration of blood and body fluids, 2) direct heating and vaporization of cellular fluid causing cells to explode, and 3) the carbonization of cells and cell fragments at high temperatures. Although initial studies have concluded that these plumes create only a malodorous nuisance, subsequent research suggests the presence of potential chemical and pathological hazards, including bacteria and virus. Most scientific research has focused on the characteristics of aerosol plumes generated through the use of electrosurgical lasers. This study investigates the ability to transfer a viral agent (P22 bacteriophage) in a viable state, from solid virus-containing agarose growth media to an airborne aerosol. The results from this study will be used as an indicator of potential exposure for health-care workers involved in ESU surgical procedures.

INTRODUCTION

During surgery many tiny blood vessels are cut, causing a loss of blood and thereby enhancing the risk of an adverse health outcome. Electrocautery surgery is used to slow down and minimize the loss of blood due to these surgical incisions by application of either electrical or laser energy to cut target tissue. During this surgical procedure, tissue cells can heat to 100° C, vaporizing their intracellular fluid and eventually rupturing the cell membrane. The rupture of the cell membrane creates an aerosol that is released into the atmosphere of the surgical suite.

Results of recent scientific research investigating the toxic components of electrocautery aerosols have initiated concern over the potential for operating room personnel to be adversely exposed during surgery (Tulikwa, 2001). Several studies have documented the presence of toxic substances in electrocautery aerosols including hydrogen cyanide, formaldehyde, blood fragments, human papilloma virus (HPV), and the human immunodeficiency virus (HIV) (NIOSH, 1988; Baggish, 1991; Hensman, 1998; Fletcher, 1998; NIOSH, 1990; Tomita, 1980). The risk of exposure is significant as the number of in- and out-patient procedures involving electrocautery surgery exceeds

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31 million per year (CDC, 2002a; CDC, 2002b).

The purpose of this study is to investigate the ability to transfer a viral agent (P22 bacteriophage) in a viable state, from solid virus-containing agarose growth media to an airborne aerosol. Vaporization of the P22-containing solid growth media is accomplished using the electrical heating element of a Bovie[®] electrocauterizer. The presence of a viable viral agent in electrocautery aerosols raises important questions concerning the potential for this surgical procedure to adversely expose unsuspecting operating room personnel to biopathogens via the inhalation pathway.

MATERIALS AND METHODS

The presence of viable viral material in electrocautery aerosols was investigated through collection of airborne aerosol generated by the electrocauterization of top agarose growth media inoculated with an identified viral product, P22 bacteriophage. The methods and materials used to perform this investigation are outlined in the following subheadings.

Growth of P22 Bacteriophage

A bacteriophage is a virus that replicates inside the cell membranes of host bacteria. Given the appropriate environmental conditions, replication of the virus continues until the bacteria's cell membrane is lysed, resulting in greater numbers of replicated virus available to infect more bacteria. In experimental procedures, evidence of virus-infected bacteria is provided by the formation of plaques or clear zones created by the lysis of bacterial cell membranes. Quantification of the viral material is accomplished by the counting of plaques, with one plaque equal to one plaque forming unit (PFU).

The virus used in this study is a P22 bacteriophage, chosen because of its relatively innocuous and robust nature. The bacterium used in this study was *Salmonella Typhimurim*, chosen because of its ability to serve as an appropriate host to P22 bacteriophage. Reconstitution of the virus and bacterium was performed using appropriate techniques outlined by the American Type and Culture Center (ATCC).

Establishment of a bacteriophage titer. Using serial dilutions, 0.1ml of bacteriophage stock solution was combined with 0.9ml of phosphate buffered solution (PBS) to a final dilution concentration of 10^{-10} . Each serial dilution was then combined with 0.1ml of salmonella stock solution and 2.5ml of molten agarose growth media. This mixture was then vortexed and poured onto sterile Petri dishes and incubated at 36°C for 24 hours. After 24 hours each Petri dish was removed from the incubator and the number of PFUs were counted, yielding a bacteriophage titer concentration of 7.5×10^9 per ml. The diluted bacteriophage stock solution associated with this titer concentration was then preserved for use in electrocautery aerosol sampling.

Electrocautery Aerosol Sampling

Three sampling runs were performed to investigate the presence of viral material in electrocautery aerosols. The same sampling methodology was performed during each run and is described in the

following paragraph.

A mixture of 0.9ml of molten top agarose growth media and 0.2ml of diluted P22 phage stock was placed in a sterile test tube and cooled, forming a plug. This plug was then placed on a sterile plate and cauterized for three minutes using a Bovie[®] 1C electrosurgical generator equipped with a Bovie[®] electrosurgical pencil (electrical heating element). Aerosolized smoke was collected under negative pressure (capture velocity equal to 80 feet per minute) through tubing placed 2cm from the combustion zone. The aerosol-containing tubing was passed through a sealed impinger with its terminal end submerged in 2.5ml of PBS trap solution. The PBS trap solution was serially diluted and combined with 0.1ml of salmonella broth and 2.5ml of molten top agarose growth media. This mixture was vortexed and poured onto sterile Petri dishes and incubated at 36⁰C for 24 hours. After 24 hours the Petri dishes were removed from the incubator and the number of plaque forming units (PFU) counted.

Experimental Controls

Three different control methods were performed during the study to determine if unwanted phage or bacteria microorganisms had contaminated the experimental procedure. If present, unwanted phage or bacteria microorganisms would invalidate the study results. The following subheadings describe the control methods performed in this study.

Control method #1: test for unwanted bacteria and phage in PBS solution trap solution. This control method was performed to test for the presence of PBS solution contaminated with unwanted bacteria or phage microorganisms. This test was performed by mixing 0.1 ml of PBS with 2.5ml of molten top agarose growth media. This mixture was then vortexed and poured onto sterile Petri dishes and incubated at 36⁰C for 24 hours. After 24 hours the Petri dishes were removed from the incubator and visually inspected for the presence of PFUs.

Control method #2: test for unwanted bacteria in phage stock. This control method was performed to test for the presence of P22 bacteriophage stock contaminated with unwanted bacteria. This test was performed by mixing 0.1 ml of P22 bacteriophage stock with 2.5ml of molten top agarose growth media. This mixture was then vortexed and poured onto sterile Petri dishes and incubated at 36⁰C for 24 hours. After 24 hours the Petri dishes were removed from the incubator and visually inspected for the presence of PFUs.

Control method #3: test for unwanted phage in bacteria stock. This control method was performed to test for the presence of Salmonella stock contaminated with unwanted phage. This test was performed by mixing 0.1 ml of Salmonella stock with 2.5ml of molten top agarose growth media. This mixture was then vortexed and poured onto sterile Petri dishes and incubated at 36⁰C for 24 hours. After 24 hours the Petri dishes were removed from the incubator and visually inspected for the presence of PFUs.

RESULTS

Table 1 below summarizes the results for both the P22 aerosol sampling runs and experimental control methods performed during this study. Each aerosol sampling run

Table 1. Results of Aerosol Sampling Runs and Experimental Controls

Aerosol Sampling			Experimental Controls		
Sampling Run No.	Result (PFU/ml)	Assumption	Control Method No.	Result	Assumption
1	3400	P22 transferred via aerosol	1	Negative for bacteria & phage	Uncontaminated PBS Solution
2	2050	P22 transferred via aerosol	2	Negative for bacteria	Uncontaminated P22 phage stock
3	3600	P22 transferred via aerosol	3	Negative for phage	Uncontaminated Salmonella stock

produced quantifiable results of P22-inoculated agarose growth media in PFUs per ml. Table 1 also shows that each control method was negative for the presence of unwanted bacteria or phage microorganisms, suggesting that the experimental procedure was not contaminated during the performance of each sampling run and the associated results are valid.

DISCUSSION

The results of this study suggest that viable viral material can be transferred via an aerosol generated by the use of an electrocauterizing unit. Even so, it is important to note that the results acquired in this study are based on a controlled experiment and do not precisely simulate the environmental conditions and behaviors present in an operating room setting. For instance, the point of aerosol collection during each sampling run was within 2cm of the organic material being cauterized. This close proximity of aerosol collection does not accurately approximate the different breathing zones encountered by the surgeons, nurses, and other personnel who are located in the surgical suite during electrocautery surgery. In addition, for each sampling run performed in this study, the length of cauterization time used to vaporize the organic material was held constant and does not simulate the intermittent cauterization pattern typically exhibited during this surgical procedure. Finally, these study results are based on the cauterization of solid agarose and it is unlikely that the burn dynamics of this media precisely simulate those of human tissue.

CONCLUSIONS

The results of this study suggest that viable infectious agents can be aerosolized during electrocautery surgery. In an effort to corroborate these results, the authors recommend that more

studies be performed evaluating the ability to transfer viral material via electrocautery smoke. Future studies should concentrate on the use of different viral agents (e.g. Hepatitis C) and the determination of virus concentrations at varied distances from the source (e.g. breathing zone distances similar to those encountered in a surgical suite). The authors also feel that the results of this study indicate the need for a smoke evacuator to be used during all electrocautery surgical procedures. It is felt that adherence to this recommendation will minimize the potential for operating room personnel to be adversely exposed to airborne infectious agents during electrocautery surgery.

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