

The Effect of Soil Accumulation on Multiple Decontamination Processing of N95 Filtering Facepiece Respirator Coupons Using Physical Methods

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ABSTRACT

The supply of filtering facepiece respirators (FFRs) may not be adequate to match demand during a pandemic. One possible strategy that has been identified is to reuse the FFR after application of a decontamination method to remove infectious material contaminating the FFR. One of the factors affecting decontamination in real world application is soil load (accumulated residues such as cells, cell debris and proteins), which can inhibit the effectiveness of the decontamination method. The objective of this study was to assess the effect of soil load on repeated decontamination of FFR coupons using energetic methods; microwave-generated steam and ultraviolet germicidal irradiation (UVGI). MS2 bacteriophage, suspended in media containing soil loads, was aerosolized and applied to FFR coupons. The coupons were cyclically contaminated and then treated with microwave-generated steam or UVGI for three series. The effect of soil load was evaluated by measuring decontamination efficacy, residual protein concentration, and UVGI penetration into the contaminated coupons. Soil load, measured as protein concentration, increased with each successive cycle of contamination. Microwave-generated steam decontamination efficacy was similar for all three cycles regardless of the soil accumulation ($p = 0.34$). UVGI decontamination demonstrated a difference in efficacy among the cycles of both the low and high soil load sample sets ($p < 0.01$). UVGI penetration into the FFR generally decreased with an increase in soil load but demonstrated a linear correlation with decontamination efficacy ($R^2 = 0.88$). Steam treatment of FFRs may be a viable decontamination technique for multiple cycle treatments, given the lack of effect of the soil load on virus inactivation efficacy. Soil load has the potential to lessen the efficacy of UVGI, but this effect can be mitigated by measuring the decrease in UVGI irradiance and compensating with increased UVGI exposure time.

Keywords: filtering facepiece respirator, N95, reuse, decontamination, UVGI, steam

INTRODUCTION

National Institute for Occupational Safety and Health (NIOSH)-certified N95 filtering facepiece respirators (FFRs) are recommended for protection against airborne transmission of infectious microorganisms (Centers for Disease Control and Prevention, 2009a; Centers for Disease Control and Prevention, 2009b). Supplies of FFRs may not be adequate to match the increased demand during a pandemic crisis (Committee on the Development of Reusable Facemasks for Use During an Influenza

Pandemic, 2006), because current guidance recommends that they be discarded after each use (Centers for Disease Control and Prevention, 2009b; Centers for Disease Control and Prevention, 2008). The Institute of Medicine (IOM) reported that demand for N95 FFRs by the health care sector alone could eclipse 90 million for a 42-day influenza pandemic outbreak (Committee on the Development of Reusable Facemasks for Use During an Influenza Pandemic, 2006). Past experiences with respiratory disease outbreaks lend support to the IOM's findings. For example, the Sunnybrook Hospital in Toronto, Canada reportedly used 18,000 FFRs a day during the 2003 severe acute respiratory syndrome outbreak causing a strain on supply (Friesen, 2003; Rubinson, 2005).

Strategies have been identified to prepare for challenges to maintaining supplies of FFRs. One strategy, the stockpiling of FFRs, has been recommended and practiced as part of pandemic influenza preparedness (Occupational Safety & Health Administration, 2008). As of December 2009, the US Strategic National Stockpile (SNS) included approximately 20 million FFRs (a combined 84.5 million were released in May and October 2009) for distribution to the healthcare sector during an influenza pandemic (Centers for Disease Control and Prevention, 2010). A second strategy, the reuse of FFRs, may extend the useful life of a disposable FFR through repeated donnings (Committee on the Development of Reusable Facemasks for Use During an Influenza Pandemic, 2006). Reuse of FFRs may result in a risk of contact transmission which could occur by touching a contaminated surface of the respirator followed by touching the eyes, nose, or mouth. Decontamination, the inactivation or removal of infectious agents through physical or chemical means, may reduce the risk of contact transmission; however, suitable decontamination protocols that do not degrade the performance of the FFR or harm the user must first be developed. The IOM suggested that the simple decontamination techniques such as microwaves and ultraviolet light should be investigated (Committee on the Development of Reusable Facemasks for Use During an Influenza Pandemic, 2006).

Ultraviolet germicidal irradiation (UVGI) and microwave generated steam treatment (hereinafter referred to as steam) of FFRs (whole or excised coupons) has been previously described for decontamination efficacy and impact on FFR fit and filtration (Bergman, 2010; Fisher, 2009; Fisher, 2010b; Heimbuch, 2009; Viscusi, 2009; Viscusi, 2007; Vo, 2009). Heimbuch et al. (2009) reported virus reduction reaching the detection limit for UVGI and steam treatment of H1N1 contaminated FFRs. Vo et al. (2009) demonstrated similar results for MS2 reduction on FFRs using UVGI. Fisher and Shaffer (2010) characterized the transmission of UVGI through FFR material and suggested a method to calculate FFR model specific doses. Bergman et al. (2010) reported no deleterious effects of both a single application of steam and UVGI on the filtration performance of the tested FFRs. Likewise, preliminary data produced in our lab demonstrated that one cycle steam and UVGI treatments of FFRs had no effect on FFR fit for the models tested (Viscusi, 2010).

Multiple (3 cycle) UVGI and steam treatments of FFRs have been evaluated for effects on filtration performance, and an investigation into multiple UVGI and steam treatments on FFR fit is being conducted within our research group. However, the virucidal efficacy of multiple UVGI and steam treatments has not been previously determined. Multiple contaminations/decontaminations of FFRs may lead to the accumulation of residual constituents of entrapped infectious aerosols. Residual organic/inorganic materials, defined here as soil load (SL), have been shown to provide physical and chemical protection to microorganisms and diminish decontamination efficacy (Ayliffe, 2000; Geiss, 1995; Johnson, 1997; Lappalainen, 2009; Martiny, 2004; Penna, 2000; Pottage, 2010; Rutala, 2008; Van Eldik, 2004). For FFRs worn by healthcare workers in a hospital setting, SLs come primarily from respiratory secretions (saliva, mucus, cellular debris, proteins, etc.) that are expelled along with microorganisms by both the wearer and nearby infected individuals while talking, sneezing coughing, and breathing (ASTM, 2010). Research in our lab has demonstrated the effects of SL in decontamination of FFR coupons (Fisher, 2009). The cleaning of FFRs to remove the SL prior to decontamination and reuse has not been investigated previously and remains a key knowledge gap.

Cleaning procedures serve to remove all or portions of the soil load and microbial contaminants. The benefits of a cleaning procedure before decontamination have been reported (Chaufour, 1999; Martiny, 2004; Penna, 2000; Vickery, 2009). But a cleaning procedure may not be practical for FFRs. Viscusi et al. 2007, demonstrated that submerging FFR in soap and water or isopropyl alcohol negatively impacted the filtration performance of FFRs. Sodium hypochlorite at 0.6% did not harm filtration

performance but presents possible health hazards of noxious chemical residues (Salter, 2009; Viscusi, 2009). Other chemical washes may be feasible except for concerns over the drying time, which would negatively impact available supplies. A minimum of 12 hours for FFRs to dry after being submerged in various solutions was reported previously (Viscusi, 2009; Viscusi, 2007).

In this study, the effect of soil buildup of aerosolized virus containing particles on UVGI and steam decontamination of FFR coupons was examined. Two SL levels were used and compared to help discern the effects of SL on UVGI and steam decontamination. Multiple cycles of contamination and decontamination were evaluated for protein buildup and removal, decontamination efficacy, and UVGI transmittance through contaminated FFR coupons. Due to its previous characterization as a test virus for FFR research, bacteriophage MS2 was chosen as a viral threat representative for this study (Fisher, 2009; Fisher, 2010a; Fisher, 2010b; Rengasamy, 2010; Vo, 2009). As the focus of this research was to determine the effects of SL on UVGI and steam and not the efficacy of the methods per se, the use of MS2 as the test virus is advantageous based on its previous characterization. Research on the effects of SL on decontamination procedures may lead to viable decontamination methods that are less affected by SL and procedures that can account for the effect of soil buildup for FFR reuse.

METHODS

Media, Virus, and Host Cells

The media, virus, and host cells, used in this research, have been described previously (Fisher, 2009). Briefly, American Type Culture Collection (ATCC) medium 271 (<http://www.atcc.org/Attachments/3600.pdf>) was used to grow *Escherichia coli* (ATCC 15597) and prepare, store, recover, aerosolize and assay MS2 (ATCC 1597-B1). The aerosol-generating medium consisted of 100% ATCC medium 271, referred to as high soil load (HSL) medium, and 1% ATCC medium 271, referred to as low soil load (LSL) medium.

FFR Selection and Coupon Preparation

The NIOSH-approved surgical N95 respirator, the 3M 1860, used for this study is comprised of multiple layers of hydrophobic and hydrophilic material. Circular coupons measuring 5 cm² were excised from FFRs using a plastic circular pattern and scissors. The six coupons for each aerosol loading (discussed below) were excised from at least two respirators. For UVGI penetration measurements, the distinct layers of the FFR (coupon) were separated into distinct layers. A layer was determined to be distinct if it separated from adjoining layers without damage to any portion of the respirator media.

Virus Loading

Respirator coupons (six simultaneously, the capacity of the aerosol system) were loaded with MS2-containing particles using the bioaerosol respirator test system (BARTS) (Fisher, 2009). Six respirator coupons (excised from at least two different respirators) were placed into separate test specimen holders and attached to the six BARTS sample ports. A titer of MS2 (approx. 10⁸ and 10⁷ plaque forming units (PFU)/ml for LSL and HSL, respectively) was suspended in the aerosol medium, placed in the Collision nebulizer jar, and aerosolized into a mixing chamber. The aerosol was pulled through each coupon with a vacuum flow rate of 4 L/min for 30 min.

Virus Recovery and Enumeration

Virus was recovered from the control and experimental coupons by agitation using a vortexer and assayed using a single agar layer method. Eight milliliters of ATCC soft agar medium 271 was placed

into glass culture tubes and incubated at 47°C in a water bath. Log-phase *E. coli* (0.5 ml) and 1 ml of the MS2 suspension were added to the culture tubes. The soft agar containing *E. coli* and MS2 was poured into an empty petri plate and mixed by swirling. The plates were allowed to harden at room temperature and placed in an incubator at 37°C and 30% relative humidity overnight. Viruses on the plates were counted on the following day, and the data for the plates containing 30 to 300 PFU were recorded.

Protein Determination

A Micro BCA™ Protein Assay Kit (Pierce Biotechnology, Rockford, IL) was used according to the manufacturer's instructions. All reagents and BSA stock solutions were freshly prepared. Following each contamination/decontamination cycle (1X, 2X, 3X), control (without decontamination) and experimental (UVGI or steam treated) coupons were placed in 10 ml of sterile-deionized water and agitated with a vortexer for 1 min. Each protein standard, control sample and experimental sample (1 ml) was mixed with 1 ml of the working reagent of the Micro BCA™ Protein Assay Kit 2 in labeled test tubes. The tubes were covered and incubated at 60°C in a water bath for 1 hr. After 1 hr, the tubes were cooled to room temperature. Absorbance (562 nm) of the protein standard and experimental solutions was measured using a spectrophotometer (U-3101, Hitachi). A calibration curve of the protein standards was constructed to determine the protein concentration of the test samples.

UVGI Penetration (Transmittance) Measurement

UVGI transmittance through the respirator layers was determined using a UV-X-25 sensor and UV-X radiometer (UVGIP Inc., Upland, CA). The layers of the FFR were placed under a low pressure mercury UV lamp (Philips 40 watt 36T5 SP) and the penetrating intensity of the light was measured. The configuration of the original FFR structure was maintained as successive layers were added between measurements. Measurements were conducted in both the interior to exterior and exterior to interior directions after each loading cycle (1X, 2X, and 3X) using particles generated from LSL or HSL medium.

UVGI and Microwave Generated Steam

Coupons contaminated with MS2 containing particles generated from LSL and HSL media were decontaminated with UVGI or steam as previously described (Fisher, 2009; Fisher, 2010b). Contaminated coupons were exposed to UVGI (2.5 mW/cm²) for 10 min on each side for a total dose of 30,000 J/m² using a low pressure mercury arc lamp (36T5 SP; a Philips 40 watt) in a biological safety cabinet (SterilGARD® III Advance°, The Baker Company Sanford, Maine). For steam treatment, contaminated coupons were placed into a steam chamber constructed from a pipette tip box as described previously (Fisher, 2009). The bottom of the chamber was filled with 50 ml of room temperature tap water. The coupons were placed on the perforated surface of the pipette tip box approximately 2.5 cm above the water level. The tip box lid, pierced with nine steam exhaust ports (approximately 4 mm in diameter), was placed on the chamber. The chamber was placed in a microwave (model R305KS, Sharp Electronics, Mawwah, NJ) and heated for 40 sec. This treatment time was used to remain within the detection limits of the enumeration assay for this steam method as previously determined (Fisher, 2009).

Loading and Decontamination Cycles

Figure 1 illustrates the process of loading and decontamination for all three cycles of a decontamination method for one level of SL. For each cycle, the experimental coupons received one additional decontamination treatment as compared to the control. Using the one cycle treatment as an example, the experimental coupons were processed once by UVGI or steam, while the control samples were left untreated. Likewise for the three cycle experiment, experimental coupons were exposed to three treatments, while control coupons underwent two cycles of decontamination. By following this process, the incremental change in decontamination efficacy among the cycles could be evaluated. It should be

noted that coupons undergoing the steam process were dried at room temperature for 45 minutes between decontamination and loading cycles.

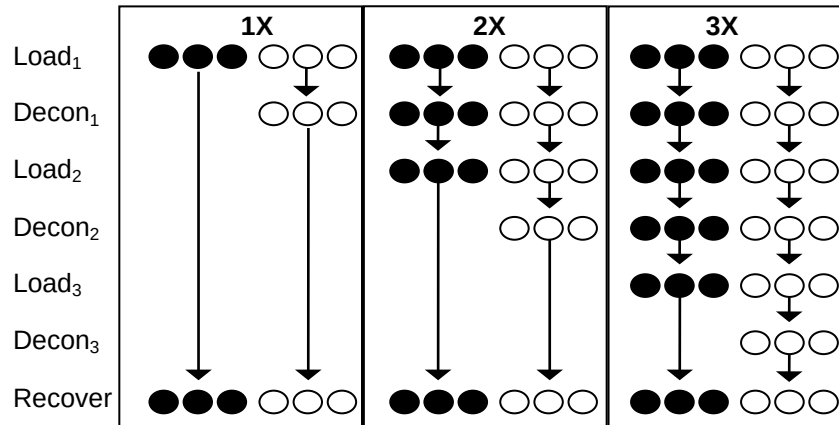


Figure 1. Schematic of the experimental design for decontamination efficacy testing of UVGI and steam. Control (●) and experimental (○) coupons were loaded with MS2 and decontaminated for one (1X), two (2X) or three (3X) cycles before the virus was recovered. For each cycle, control coupons (n=3) received one less decontamination treatment compared to experimental coupons (n=3).

Respirator coupons were loaded with virus containing particles generated in LSL or HSL medium as described above. For one cycle of loading and decontamination, six coupons were loaded with approximately 10^6 PFU of MS2. Upon loading, three coupons were placed into the recovery buffer to serve as the loading control. The remaining three coupons were decontaminated with either UVGI or steam as described above, after which the coupons (termed experimental) were placed in the recovery buffer for enumeration. For the two cycle experiments, six coupons were loaded with MS2, decontaminated, and again loaded with aerosolized MS2. After the second loading with MS2, three coupons were placed in the recovery buffer to serve as the control. The three remaining coupons (experimental) were decontaminated before placement in the recovery buffer. For the three cycle experiments, six coupons were loaded with MS2 and decontaminated for two cycles. After a third loading, three coupons were placed in the recovery buffer (control), and three coupons (experimental) were again decontaminated before placement into the recovery buffer.

Data Analysis

The antiviral activity of the UVGI and steam treatment methods of the FFR coupons was determined by calculating the $\log_{10} N/X$ where N is the titer of viable MS2 recovered from the control coupons and X is the titer of the viable virus recovered from the treated coupons. A single factor analysis of variance (ANOVA) with replication (95% confidence level) was performed, using EXCEL (Microsoft Office 2003), to determine statistical significance of the data among the LSL and HSL samples and loading/decontamination cycles. A two-tailed standard T-test was used to test between two sample groups where appropriate.

RESULTS

Figure 2 demonstrates the increase in protein concentration on contaminated FFRs with each loading with HSL droplet nuclei. The control sample set demonstrated an increase from 76 $\mu\text{g}/\text{cm}^2$ of protein after one loading to 326 and 442 $\mu\text{g}/\text{cm}^2$, for the second and third loadings, respectively. The decontaminated coupons demonstrated similar trends with protein concentrations measuring 177, 252, and 442 $\mu\text{g}/\text{cm}^2$ for steam and 80, 254, and 461 $\mu\text{g}/\text{cm}^2$ for UVGI corresponding to the first, second, and third loading/decontamination cycles, respectively.

The three cycles of steam decontamination of FFRs achieved log reductions (LRs) of 2.5, 2.1, and 3.0 for LSL and LRs of 2.5, 2.5, and 2.1 for HSL for the first, second, and third cycles, respectively (Fig. 3). The ANOVA results did not demonstrate statistical difference among both the six LSL and HSL sample sets ($p=0.34$) or within the LSL ($p=0.10$) and HSL ($p=0.60$) sample sets tested individually.

UVGI decontamination efficacy generally decreased with an increase in soil load (Fig. 4). Log reduction of MS2 in the LSL sample set measured 2.8, 2.8, and 2.1 for the 1X, 2X, and 3X loading/decontamination cycles, respectively with the second and third cycles demonstrating a statistical difference ($p=0.02$) using the t-test. LRs of the UVGI decontamination of the HSL sample set measured 1.9, 1.1, and 0.9 ($p<0.01$) for the first, second, and third cycles. The reduction of viable MS2 in both LSL and HSL medium demonstrated correlation ($R^2=0.88$) with the corresponding UVGI penetration values for each of the loading/decontamination cycles (Fig. 6).

The percentage of UVGI penetrating to the interior layer of the FFR coupons decreased with an increase in protein content (Fig. 5). Penetration levels for the LSL sample set measured 8.7, 10.1, 7.0%, while the HSL samples scored penetration values of 2.1, 1.1, and 0.6% for successive loadings.

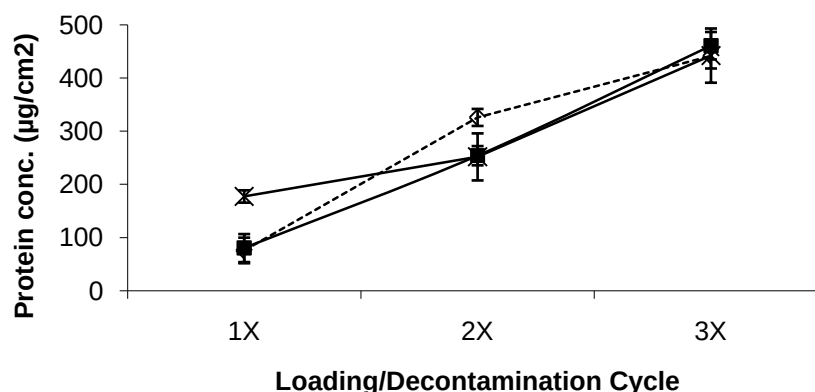


Figure 2. Average protein concentrations ($\mu\text{g}/\text{cm}^2$) on FFR coupons loaded with HSL droplet nuclei for successive cycles of loading without decontamination (◇), with steam (×), and UVGI (■).

DISCUSSION

The persistence of SL on reprocessed dental and medical equipment has been examined under actual and laboratory conditions (Bannon, 2005; Chan-Myers, 1997; Clery, 2003; Johnson, 1997; Lappalainen, 2009; Smith, 2005; Vickery, 2009; Zhong, 2009). Smith et al. (2005) found residual protein (from 0.5 μg to 63.2 μg) on all 220 sampled endodontic files that were cleaned and sterilized and deemed ready for reuse. Lappalainen et al. (2009) demonstrated the buildup of protein on gastrointestinal biopsy forceps under laboratory conditions. Zhong et al. (2009) modeled the buildup of protein on reused

medical devices. In alignment with previously described occurrences of soil buildup, the test system used in this study provided a method to increase the SL content of the samples with each successive loading (Fig. 2). Figure 2 demonstrates that the multiple steam and UVGI decontaminations of the contaminated coupons did not remove the SL. The inability of steam sterilization to reduce SL has been demonstrated in previous studies and removal of SL via UVGI irradiation is not expected (Bannon, 2005; Clery, 2003; Smith, 2005).

The decontamination efficacy of steam was not affected by the level of protective residue from the LSL and HSL media or from successive MS2 loadings (Fig. 3). Moist heat, more commonly applied in the form of pressurized saturated steam (autoclave), is less affected by SL than other sterilization methods (Rutala, 2004). Unfortunately, autoclaves severely degraded the FFR and thus less aggressive steam methods are necessary for FFR decontamination (Viscusi, 2007). Although the steam method may not reach the level of sterilization seen with autoclaves, the negligible effects of the SL should be comparable. Other studies have demonstrated comparable decontamination of clean and residue-challenged surfaces using thermal methods (Johnson, 1997; Van Eldik, 2004).

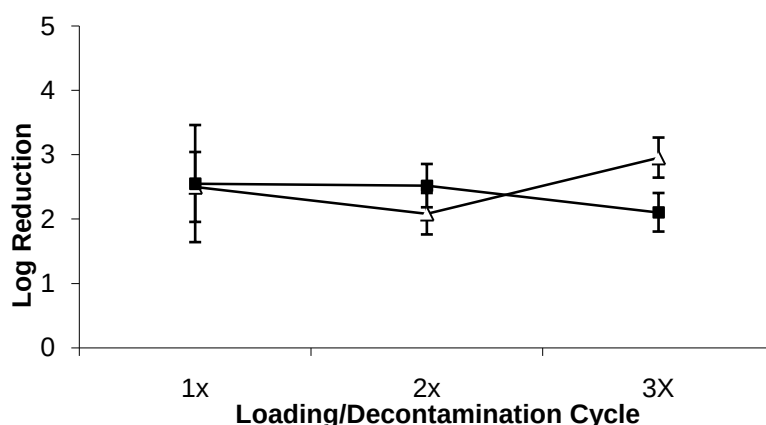


Figure 3. The log reduction of MS2 for successive loading/steam decontamination cycles. Droplet nuclei containing MS2 was generated from HSL (■) and LSL (▲) media.

In contrast to the steam, UVGI was less efficient at MS2 inactivation when the level of SL increased. Absorbed by proteins and other organic materials, UVGI penetration through SL is problematic (Lai, 2004; Templeton, 2005). Figure 4 shows a marked difference in the protective effect of HSL in comparison to LSL and between the first and third cycles regardless of the medium. The results suggest that a cleaning procedure may be necessary to allow UVGI to be effective in decontaminating FFRs.

A cleaning procedure may negate some of the benefits of using UVGI, a dry method that is not aggressive to the substrate and should not produce potentially hazardous chemical residues. A mechanism discussed in Fisher and Shaffer (2010b) may permit the use of UVGI for the decontamination of FFRs despite the presence of a protective residue, rendering cleaning as unnecessary. The measured UVGI penetration to the filtering layer of the FFR coupon decreased with increasing SL produced by the aerosol media, HSL and LSL, and successive loadings (Fig.5). Plotting the UVGI penetration against the efficacy for all samples tested reveals a linear correlation similar to the UVGI dose response discussed in Fisher and Shaffer (2010b). Although Fisher and Shaffer (2010b) did not measure the penetration of UVGI through levels of soil load in cyclic loadings, the mechanism to determine dose for residue free FFRs is also applicable to contaminated filters as demonstrated in Figure 6. UVGI doses can be increased to account for expected levels of SL.

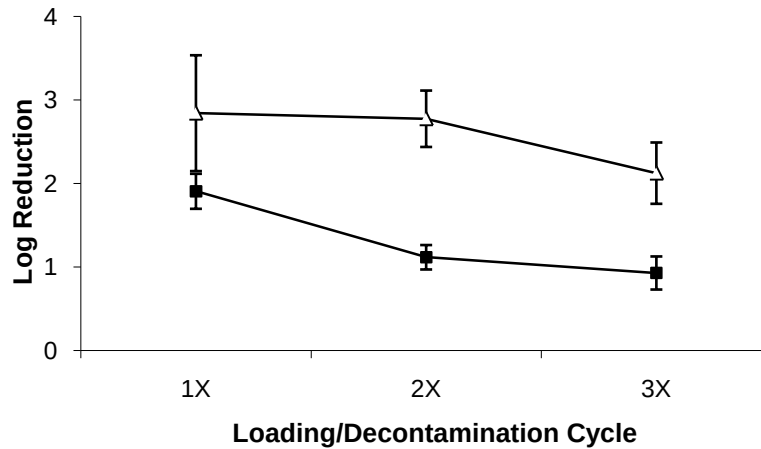


Figure 4. The log reduction of MS2 for successive loading/UVGI decontamination cycles. Droplet nuclei containing MS2 was generated from HSL (■) and LSL (Δ) media.

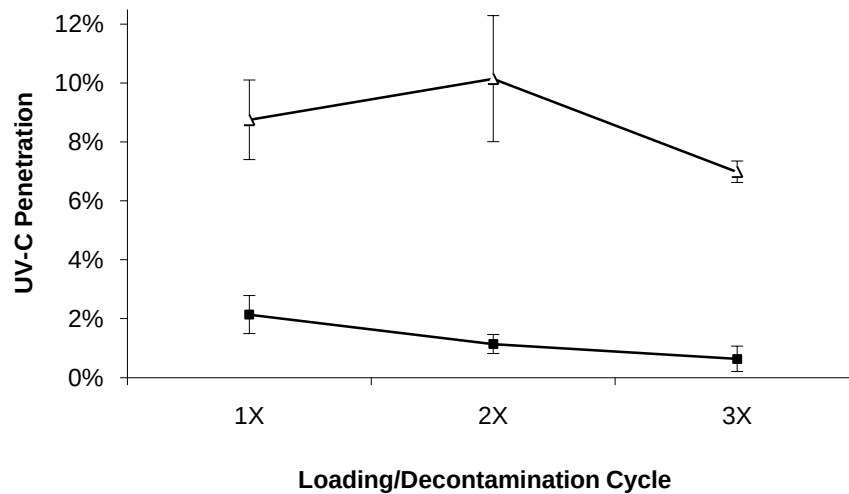


Figure 5. The average percentage of the surface UVGI reaching the filtering medium of the FFR coupons for successive loadings with HSL (■) and LSL (Δ) droplet nuclei.

The severity of the UVGI and steam methods used in this study was derived from previous efforts to provide inactivation rates within the limits of detection (Fisher, 2009; Fisher, 2010b). This was necessary to increase the sensitivity in determining the effect of SL. The methods were not designed to provide complete inactivation of MS2 and should not be critiqued on decontamination efficacy. The methods are promising considering the protein concentrations ranged from approximately 80 to 480 $\mu\text{g}/\text{cm}^2$ protein as measured by BCA assay. Although actual field data is lacking for the level of SL of contaminated FFRs, the protein concentrations used in this study greatly exceed reported values for other medical equipment and challenge standards for laboratory testing (Chan-Myers, 1997; Lappalainen, 2009; Smith, 2005).

More research is required before decontamination methods for FFRs can be utilized. Other microorganisms, SL, and FFR types should also be studied. Although this study examined decontamination without pre-cleaning, research is necessary to investigate the effects of physical and chemical SL removal. Further research on decontamination procedures that are less affected by soil load and procedures that can account for the effect of soil buildup may lead to viable decontamination methods for FFR reuse.

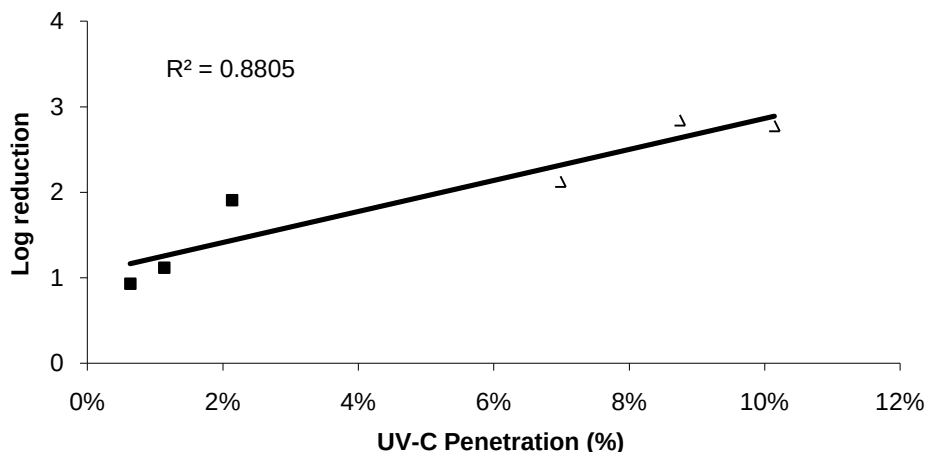


Figure 6. The decontamination efficacy of UVGI treatment as a function of the percentage of UVGI reaching the filtering medium of the FFR coupons loaded with MS2 in HSL (■) and LSL (Δ) droplet nuclei.

CONCLUSIONS

Steam treatment of FFRs may be a viable decontamination technique for multiple cycle treatments, given the lack of effect of the SL on virus inactivation efficacy. Soil load has the potential to lessen the efficacy of UVGI, but this effect can be mitigated by measuring the decrease in UVGI irradiance and compensating with increased UVGI exposure time.

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Disclaimer

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