Smell Fresh Arizona, LLC.

Air Purification Systems

The Science

This document has been prepared specifically to address the question:

Will these devices work in the prevention of the spread of SARS-CoV-2 (the novel coronavirus)?

Preface

To help contain the spread of the Coronavirus, owners and operators of commercial spaces have the responsibility to take precautionary measures, and mount an effective response if you believe or know a building has been exposed. That said, businesses wanting to remain open during this COVID-19 pandemic, or bring employees back to work, or open their doors to the public are incredibly burdened by what approach to take to responsibly and practically keep their workspaces safe for employees and customers.

In this day and age of the internet, we can suffer from information overload, as well as paralysis by analysis. This is especially true when you can find conflicting information to support, or dispel, most any claim. Knowing this, we at Smell Fresh Arizona feel our best approach to support our product claims, is to offer you an understanding of the science of viruses and the scientific mechanisms of how they are neutralized outside of a human host. With this knowledge, we trust you'll recognize the power of our technology in your overall plan to minimize risk of COVID-19 exposure at the workplace for employees and customers.

THE BASIS FOR MUCH OF OUR SCIENTIFIC CLAIMS COMES FROM RESEARCH ARTICLES FROM THE NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION: https://www.ncbi.nlm.nih.gov/

What is a virus?

Generally speaking, viruses are submicroscopic infectious agents that are usually regarded as nonliving extremely complex molecules that typically contain a protein coat surrounding an RNA or DNA core of genetic material but no semipermeable membrane, that are capable of growth and multiplication only in living cells of a host, and that cause various important diseases in humans, animals, and plants. (ref.1)

While seemingly a complex, and overly scientific explanation, we in the general public in our desire to understand how to stop its spread, and not get infected ourselves, need to understand two fundamental points from this explanation of a virus:

1. It is only capable of growth in living cells.
2. It remains an active threat in its protein or lipid coating.
    a. **SARS-CoV-2 (Coronavirus) rely on a protective lipid coating, and are the easiest type to deactivate.**

** SARS-CoV-2 is the name of the virus that causes the coronavirus disease COVID-19.*

What to make of these two fundamental points in stopping the spread of a virus, or more specifically, the Coronavirus?

"It is only capable of growth in living cells."

Let us first understand what makes viruses such a threat to people, and why we don't have a catch all medical cure for all or most viruses. For starters, they are such small molecules, that they cannot be individually seen by light microscopy available in most labs. Furthermore, they mutate so a vaccine for one mutation may not work on another. SARS-coV-2 is a specific mutation of a broader class of Coronaviruses, which has recently found its way into humans, and has caused this current pandemic.

Let us next understand why or how it spreads among people, especially given the quote: "it is only capable of growth in living cells". Once a person becomes infected, they are a host to the virus. The virus now has living cells to multiply and thrive in. The virus can attach itself to all of your living cells, so when you breathe, sneeze, cough, or rub your eyes, your secretions will contain the virus. With nothing around the virus molecules, or as these secretions dry out, these virus molecules can cease to be a threat. HOWEVER, your saliva, mucus, tears, and oils from your skin can provide protective coverings for the virus molecules to remain active for days, particularly on hard surfaces like glass, metals and plastics. **Some other points to note:**

- A virus can be active in your body before you feel the symptoms.
- A virus is obviously active while you know you have the symptoms.
- Provided you survive, your body's immune system will eventually "kill off" the virus.
- You can receive transmission from taking a breath of air that was expelled by someone infected.
- You can receive a transmission from touching biomatter deposited on a surface from an infected person, typically only if you then touch your eyes, nose, or mouth.

** While acknowledging viruses are regarded as "nonliving", we'll often reference some form of expression of "killing" viruses to imply a destruction of the virus's molecular structure.*

Two typical transmission scenarios are:

- An infected person coughed or sneezed on a surface. That surface was then touched by someone else. That person then rubs their eyes or nose, or touches something that they then put ingest.
- An infected person is talking close enough to another person for them to breathe in a breath of air expelled by the infected person. When a person breathes out, their breath is rich in moisture (water molecules) that can carry the virus over to another person's inhalation, and/or possibly be deposited on a hard surface.

So how do we stop its spread in knowing that "it is only capable of growth in living cells"?

The common methods are:

- Practice social distancing: 6 feet is the common standard right now. Do not get too close to other people, and don't shake hands.
- Avoid touching surfaces recently touched by people who could potentially be infected.
    - If you go out into the general public, you should wash your hands outside your home before entering, or at least immediately upon entering your home.
- Clean surfaces: wipe surfaces with disinfectants touched by other people who could potentially be infected.

"It remains an active threat in its protein or lipid coating."

Understanding this quote, is what enables us to clean and neutralize the virus outside a host.

So what to clean with?

In understanding that a virus only **"remains an active threat in its protein or lipid coating"** gives us a vital understanding of how to kill or neutralize them outside of a host body. Inside a person, it's hard for medical science to keep up with treating infected people. Outside of a person, the science, available technology, and chemicals are quite stout in eliminating viruses.

A virus's protein or lipid coating is easily pierced rendering the virus molecule "killed" or inactive, inert matter. Outside of a host, a virus is a relatively weakly structured molecule that can be broken down relatively easily. Certain chemical-based products break open the viruses outer coating and its internals fall apart, effectively "killing" the virus. What cleaners or mechanisms do this?

- **Soap**
- **Alcohol based cleaners (often hand sanitizers)**
- **Quaternary cleaners**
- **Oxidizers**
- **UV-C Light Radiation**
- **Ions**

Let us further examine how each chemical or mechanism neutralizes viruses outside of a host.

Soap

Soap is very effective in two ways. First, soap is a surfactant (most soap products are surfactants). Surfactant molecules contain a lipophilic (fat-loving) end that attaches to grease and dirt, and a hydrophilic (water-loving) end which makes the molecule dissolve in water. Water alone can be good at cleaning soiled surfaces, but we know soapy water is better. In reducing the surface tension of water, soapy water lets the water molecules work their way into smaller holes or pores of surfaces invisible to the human eye; thus making soapy water better than plain water in removing loose matter from objects.

Secondly, soap dissolves the fatty membrane (lipid) that holds the virus together. Given soaps are often made from fat-like substances known as amphiphiles, the soap molecules compete with the lipids holding the virus together. The cell is then broken up, becoming inert, harmless matter.

Alcohol based cleaners

Most hand sanitizers are alcohol-based cleaners. Alcohol also breaks down the protein or lipid coating of a virus molecule. The CDC suggests that alcohol-based cleaners are not as effective as soap for two reasons:

- The concentration may not be high enough. Generally speaking, hand sanitizers should be at least 70% concentration levels of alcohol for best effectiveness.
- If you do not remove the greases and soil on your hands or surfaces, the virus molecule could "hide" in these oils on your hands. Soap is more effective at removing oils and soil from your skin and other surfaces.

All considered, soap is a better option, but alcohol and alcohol-based sanitizers can also be effective.

Quaternary cleaners

Quaternaries are among the most common ingredients in common disinfectants. The most common quaternary ingredient is a form of **ammonium chloride**. If you are going to buy a common disinfectant, we strongly recommend you look for this active ingredient on the product labeling.

Quaternaries or "Quats" are cationic (positively charged) ions that disinfect surfaces by binding to the negatively charged protein or lipid coating of viruses, thus breaking down the virus molecule. Most suppliers and manufacturers describe using their product on surfaces "like

laying down a bed of spikes that pierce the outer coating of viruses or outer membranes of microorganisms on contact".

These cleaners are very effective, and are some of the most common disinfectants used in hospitals and restaurants.

Oxidizers

Common and familiar oxidizing disinfectants are: chlorine, bleach, and hydrogen peroxide. We've all used these to clean our homes. Some not so familiar oxidizers are ozone, fluorine, hydroperoxides, and hydroxy groups. Oxidizers in general are highly reactive molecules that attach themselves, or an oxygen atom or electron to other molecules. In doing so, they change the molecular structure of that other molecule. Viruses are highly susceptible to oxidation. It's outer protein or lipid is easily oxidized, breaking down the molecular structure of the virus molecule.

While they are great disinfectants, some downsides with some oxidizers is they can be highly corrosive to surfaces, harmful to people if ingested, and can "bleach" or stain some materials.

**** Our devices have a special titanium dioxide coating (TiO2) that when exposed to a light source, form hydroxyl and other hydroperoxide radicals. These radicals rank as some of natures most powerful sterilizing agents.***

UV-C Light

Light is electromagnetic radiation that travels at various wavelengths, with a portion of its spectrum visible to the human eye. In the visible light spectrum, different wavelengths are perceived as different colors. Infrared has long wavelengths, and ultraviolet has short wavelengths. Long wavelengths of light can go around virus molecules and not affect them. Short wavelengths of light can "hit" virus molecules. Coronaviruses are roughly spherical and average around 100 nm in size. UV light is defined as light ranging from 10 to 400 nanometers (nm). Germicidal lamps operate in a subcategory of ultraviolet light defined as UV-C with a spectrum ranging from 100 to 280 nm. With electromagnetic radiation at wavelengths roughly the size of the coronavirus molecule, UV-C light radiation can hit, penetrate and disrupt the virus's lipid bilayer envelope and RNA rendering the virus inert and harmless.

**** Our devices have a special germicidal UV-C lamp in them, that have been lab tested against bacteria, viruses, and fungi (mold).***

***** Note, we do not recommend UV-C lights in areas directly visible to people, as it can damage your eyes. This is why we install our systems in areas in HVAC systems or areas not in common lines of sight of people.***

Ions

Ions are atoms or molecules that have a net electrical charge. We've learned from childhood that negatively charged thing can be attracted to positively charged things. Hence the expression, "opposites attract". Most ion generators work by charging oxygen or other molecules in the air. These charged molecules will then be attracted to oppositely charged molecules. This scientific phenomenon can be used to fight airborne viruses in two ways. For starters, when two molecules combine, they often form a new molecule. A dangerous molecule could be changed into a harmless molecule. Secondly, as charged molecules in the air start coagulating or sticking together, they eventually become heavier than air. This added density causes particles to become heavier than air, falling to the ground or other surfaces to later be cleaned up. The benefit being: they are no longer airborne particles we can breathe.

**** Our devices come equipped with ion generators.***

What did we learn from this reading so far?

- **How viruses can survive outside the human body.**
- **How viruses are transmissible from person to person.**
- **How viruses are weak and easy to destroy outside the human body, and by what mechanisms.**

Our Technology

Let us circle back to the fundamental basis for this document:

Will these devices work in the prevention of the spread of SARS-CoV-2?

Why the lengthy leadup to answering this fundamental question?

Mostly to point out that even though this virus has wreaked havoc on the population on a global scale due to its ability to spread easily, it's actually a relatively weak molecule outside of a host, and can be neutralized with something as simple as soap and water. That said, it is not a stretch to now understand how our Air Purification Systems can help reduce the spread of the Coronavirus in your place of business.

The Smell Fresh Arizona, LLC. Air Purification System incorporates three proven virus killing technologies in one device: UV-C Light, Photocatalytic Oxidation, and Ion Generators.

UV-C Lamps – Proven Virus Killer!

Titanium Dioxide (TiO2) coated screen (Photocatalytic Oxidation) – Proven Virus Killer!

Ion Generators – Proven Virus Killer!

So… will these devices work in the prevention of the spread of SARS-CoV-2?

The short answer:

"The consensus of the scientific community in the testing of the three technologies included in our Air Purification Systems is that they are proven virus killers, and CDC guidelines supports the use of UV light sterilization systems in infectious disease control and prevention plans."

The long answer:

The consensus of the scientific community for the last 30+ years has conclusively proven that UV-C light, Photocatalytic Oxidation from TiO2 coatings, and ion generators are effective to varying degrees at neutralizing microorganisms and specifically RNA viruses (of which the SARS-CoV-2 is categorized).

Referenced testing reports or studies herein demonstrate the following:

Our UV-C lamp specifically has been lab tested against the following lipid bilayer structured or RNA type viruses with up to 99.99% kill rate:

- Influenza
- Bacteiophage
- Poliovirus 1
- Hepatitis B Virus

Our titanium dioxide coating has been tested specifically on the original SARS-CoV-1 strain back in 2004 with 100% inactivation/decomposition under UV radiation.

Ion generators have been lab tested with up to 97% inactivation rate of airborne viruses, and 100% prevention rate from airborne infection of various viruses tested on guinea pigs.

All said, for legal purposes, we cannot make the claim that our Air Purification Systems work against the SARS-CoV-2 virus strain as they have not been specifically tested against the SARS-CoV-2 virus strain.

Current CDC guidelines have provided that other known virus killing chemicals and devices should be used in preventing the spread of the coronavirus strain SARS-CoV-2. Given the scientific understanding and evidence we have offered, we hope you will conclude that science supports our Air Purification Systems may help in reducing the risk of the spread of the coronavirus disease in your place of business.

A personal message from our owner on the subject of testing…

This is an interesting time we are in. We are faced with a Global Pandemic. We literally have thousands of research studies over the last 30+ years on how to neutralize a virus outside of a human host, yet we can make no such legal claim. We are not claiming to have reinvented the wheel, or to have some proprietary, super virus killer. We are simply offering proven, decades old technologies to our fellow citizens to help prevent the spread of the Coronavirus. There are many manufacturers of devices that incorporate one of or more of these technologies that could be great in helping to stop the spread of the coronavirus, yet none of us can legally make these specific claims because these specific products have not been specifically tested against SARS-CoV-2, and will likely never be tested by the EPA.

From the United States Attorney General's Office on down, all law enforcement agencies only allow for the claim of a SARS-CoV-2 disinfectant if it has been tested and approved as an EPA List-N product. The EPA only tests chemicals, not devices. From the EPA website:

"Unlike chemical pesticides, EPA does not routinely review the safety or efficacy of pesticidal devices, and therefore cannot confirm whether, or under what circumstances, such products might be effective against the spread of COVID-19. Accordingly, List N only includes surface disinfectants (chemicals) registered by EPA and does not include devices."

An analogy: Does a gun manufacturer have to prove each model gun it manufactures is lethal to humans? No. It is well understood that a bullet discharged from a gun at a high velocity is lethal to humans. We don't need to test each new gun model for lethality, because of our understanding of its mechanism to be lethal. Why then must we specifically test a device to neutralize SARS-CoV-2 to make a legal claim, when it includes a technological mechanism with decades of research proving its effectiveness in inactivating RNA type viruses?

We live in a highly litigious and political environment, where only billion-dollar corporations have the resources to conduct expensive testing, and have the political influence to shape laws to make legal claims. Consider that only pharmaceutical manufacturers can make claims of drugs curing, treating or preventing diseases as medicines, while herbal manufactures can make no such claims. Similarly, because viral molecules cannot be seen under microscopes in most labs, testing of any product against SARS-CoV-2 is incredibly expensive and out of reach by most smaller companies able to manufacture devices with scientifically proven technologies.

It is unfortunate that at this time of our greatest need for every countermeasure possible, the general public is being deprived of such readily available technology to combat the coronavirus, because companies like ours have to tread lightly in offering our products in this highly litigious and political environment.

In deciding whether or not our devices are for you in your place of business, you can find online hundreds of news articles of how hospitals have used UV lights mounted on robots to sterilize rooms and hallways; how New York City is using UV light to disinfect subway cars; and how Amazon is running packages under UV light stations for the safety of their employees and consumers. I think we can trust these large, billion-dollar institutions aren't doing so on loose claims. Us little guys can follow their lead. While you don't want to use UV light in occupied spaces, you can trust that UV light is a powerful disinfectant that can kill airborne germs that pass through it, as would be the case with our HVAC mounted Air Purification Systems, with the added benefits of our photocatalytic oxidation and ion generator technologies.

We'll leave you with these parting words:

Both the Center for Disease Control and the World Health Organization both recognize the use of germicidal devices in the reduction of the spread of the coronavirus.

Our devices and coatings are not intended to replace or supersede CDC recommended practices, they are intended to compliment these practices as added safeguards for your employees and customers.

The following pages include reference links, materials and test data supporting our three virus killing technologies in our Air Purification Systems.

Whatever you decide, Godspeed.

- ***Michael Samora***
Owner/ Chief Engineer
Smell Fresh Arizona, LLC.

References:

Coronavirus

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UV light

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Other

https://www.cdc.gov/niosh/nioshtic-2/20034387.html

https://www.cdc.gov/infectioncontrol/pdf/guidelines/disinfection-guidelines-H.pdf

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Smell Fresh Arizona - Air Purification Systems

Smell Fresh Arizona has partnered with leading manufacturers to bring Advanced Oxidation Technology into your home and office. Read more about the testing of this advanced technology!

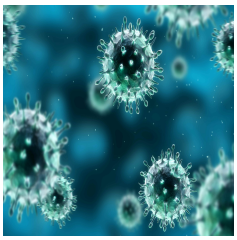
ADVANCED OXIDATION TEST RESULTS 2000-2015

This Advanced Oxidation Technology was developed over 20 years ago. Over 1 million hydroxyl/ hydro-peroxide generators are in use around the world. This technology is in use by many Fortune 500 companies for use in medical, food, military, residential, commercial, marine, hospitality and government, etc. Our suppliers products have been tested and/or approved or registered by:

	•Chinese Government	•U.S. Government
•U.S. Military	•Japanese Government (TV commercials)	•European Union
•Electric Power Research Institute	•Canadian Government	•USDA & FSIS

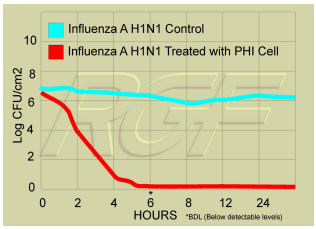
In addition, this technology has been specified in the Norovirus & MRSA protection plan of America's largest restaurant chains, hotel chains, theme parks, cruise lines, public schools and hospitals. The following is a summary of some of the testing and studies performed by third party independent labs and universities of some of our supplier's devices.

* While proven germ killers, we cannot make claims as medical devices.

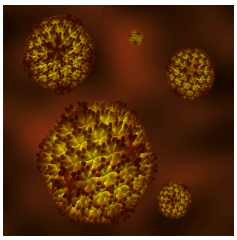


H1N1 (Swine Flu)

Kansas State University has completed preliminary testing on Photohydroionization and Reflective Electromagnetic Energy technologies with **99+% inactivation of H1N1 Swine Flu on a stainless steel surface.** Further tests are scheduled. 2009 H1N1 (referred to as "swine flu" early on) is a new influenza virus causing illness in people. This new virus was first detected in people in the United States in April 2009. This virus is spreading from person-to-person worldwide. On June 11, 2009, the World Health Organization (WHO) signaled that a worldwide pandemic of the 2009 H1N1 flu was underway. Spread of 2009 H1N1 virus is thought to occur in the same way that seasonal flu spreads. Flu viruses are spread mainly from person to person through coughing or sneezing by people with influenza. Sometimes people may become infected by touching items – such as a surface or object – with flu viruses on it and then touching their mouth or nose.

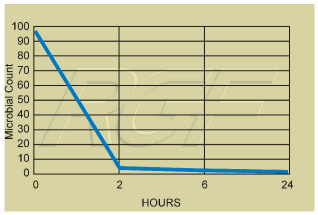


Tested by Kansas State University Inactivation Rate **99+%**

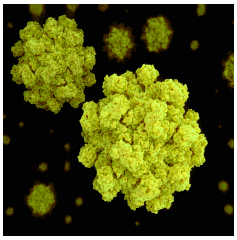


Avian influenza (Bird Flu)

Avian influenza is an infection caused by avian (bird) influenza (flu) viruses. These influenza viruses occur naturally among birds. Of the few avian influenza viruses that have crossed the species barrier to infect humans, H5N1 has had the largest number of detected cases of severe disease and death in humans. Source CDC Center for Disease Control and Prevention



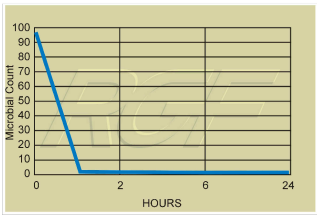
Tested by Kansas State University Inactivation Rate **99+%**



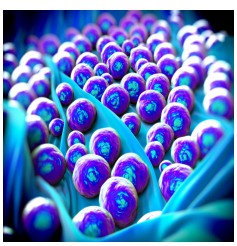
Norwalk Virus

Noroviruses are a group of related, single-stranded RNA, nonenveloped viruses that cause acute gastroenteritis in humans. Noroviruses are highly contagious and as few as 10 viral particles may be sufficient to infect an individual. 50% of all food-borne outbreaks of gastroenteritis can be attributed to noroviruses. Chicago schools realized a 20% improvement in attendance after installing RGF's PHI Technology.

Source: CDC Centers for Disease Control and Prevention

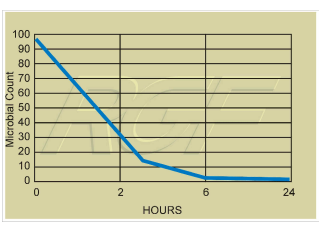


Tested by Midwest Research Institute Inactivation Rate **99+%**



Methicillin Resistant *Staphylococcus aureus*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a type of bacteria that is resistant to certain antibiotics. These antibiotics include methicillin and other more common antibiotics such as oxacillin, penicillin and amoxicillin. RGF participated, along with a major hospital, in a two year study evaluating PHI Technology which resulted in a 33.4% reduction in infections. Source: CDC Centers for Disease Control and Prevention

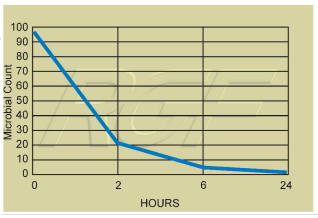


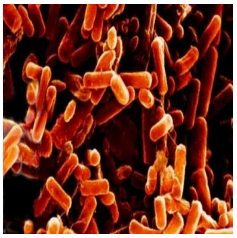
Tested by Kansas State University Inactivation Rate **99+%**

Streptococcus Sp.

Group *A Streptococcal* (strep) infections are caused by group *A streptococcus*, a bacterium responsible for a variety of health problems.
Source: U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

Tested by Kansas State University Inactivation Rate **96+%**

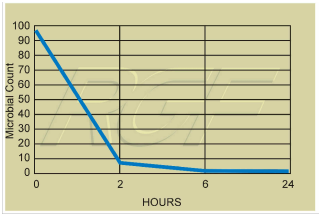


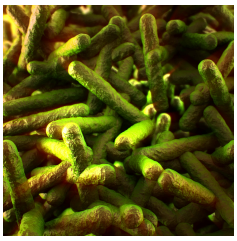


Pseudomonas Sp.

The bacterial genus *Pseudomonas* includes plant pathogenic bacteria such as *P. syringae*, the opportunistic human pathogen *P. aeruginosa*, the ubiquitous soil bacterium *P. putida*, and some species that are known to cause spoilage of unpasteurised milk and other dairy products.

Tested by Kansas State University Inactivation Rate **99+%**

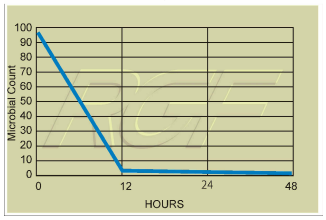


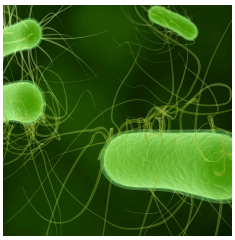


Listeria

This is a Gram-positive bacterium, motile by means of flagella. Some studies suggest that 1-10% of humans may be intestinal carriers of *L. monocytogenes.*
Source: U.S. Food and Drug Administration

Tested by Kansas State University
Steris Labs
KAG / Eco Labs Inactivation Rate **99+%**

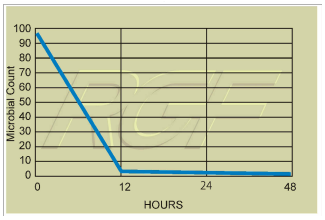


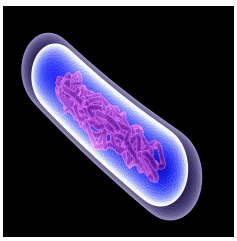


Escherichia coli

Escherichia coli, usually abbreviated to *E. coli,* discovered by Theodor Escherich, a German pediatrician and bacteriologist, is one of the main species of bacteria that live in the lower intestines of mammals, known as gut flora.
Source: CDC: Center for Disease Control and Prevention

Tested by Kansas State University Inactivation Rate **99+%**

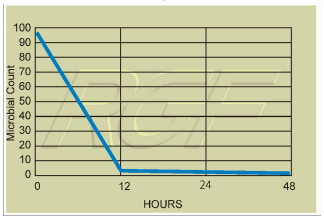


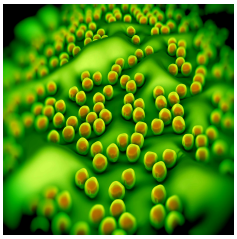


Bacillius Globigii

Bacillus globigii lives in soils around the world and can readily be found in samplings of wind-borne dust particles. It is also known as *Bacillus subtilis*, its more modern name.
Information source: CDC Center for Disease Control and Los Alamos National Laboratory

Tested by Kansas State University Inactivation Rate **99+%**

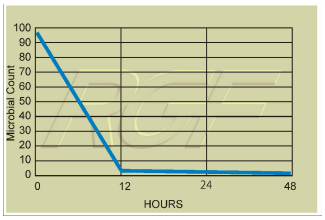


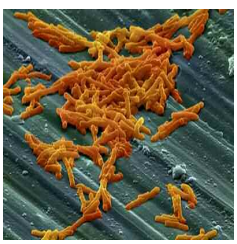


Staphylococcus Aureus

Staphylococcus aureus, often referred to simply as "staph," is a bacteria commonly found on the skin and in the nose of people. Person-to-person transmission is the usual form of spread and occurs through contact with secretions from infected skin lesions, nasal discharge or spread via the hands.
Information source: CDC Center for Disease Control and FDA (Food and Drug Administration)

Tested by Kansas State University Inactivation Rate **99+%**

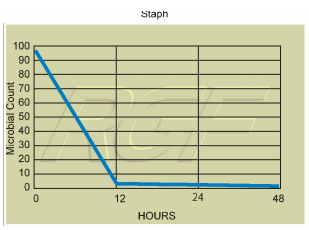


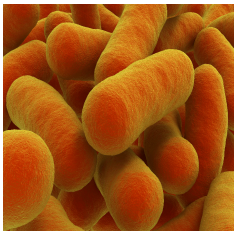


Clostridium difficile (C-Diff)

Many hospitals have been waiting for more information on C-Diff bacteria as it may be as big a problem or bigger than MRSA. Independent university studies tested RGF's REME Technology with 99% kill rate.
Source: CDC Center for Disease Control and Prevention

Tested by Kansas State University Inactivation Rate **99+%**

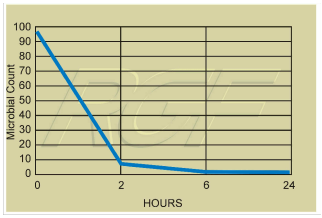


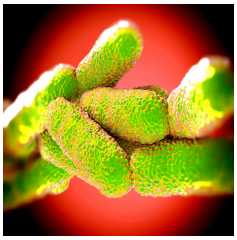


Tuberculosis

Tuberculosis typically attacks the lungs, but can also affect other parts of the body. It is spread through the air when people with infection cough, sneeze, or otherwise transmit their saliva through the air.[2] Most infections are asymptomatic and latent, but about one in ten latent infections eventually progresses to active disease which, if left untreated, kills more than 50% of those so infected. Source: Health and Industry

Tested by Kansas State University Inactivation Rate **99+%**

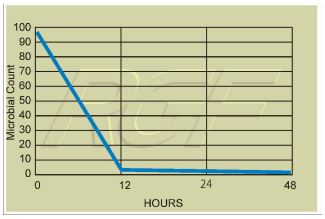


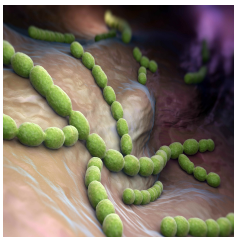


Legionella

Legionella is common in many environments, with at least 50 species and 70 serogroups identified. The chemical composition of these side chains determine the nature of the somatic or O antigen determinants, which are essential means of serologically classifying many Gram-negative bacteria. Information source: CDC Centers for Disease Control

Tested by Kansas State University Inactivation Rate **99+%**



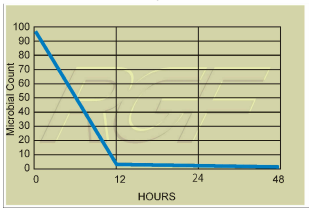


Streptococcus Pneumoniae

S. pneumoniae is an exclusively human pathogen and is spread from person-to-person by respiratory droplets, meaning that transmission generally occurs during coughing or sneezing to others within 6 feet of the carrier. Health experts estimate that more than 10 million mild infections (throat and skin) like these occur every year.

Information source: CDC Centers for Disease Control

Tested by Kansas State University Inactivation Rate **99+%**



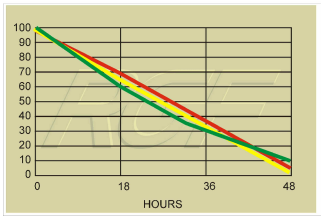
Mold/Yeast

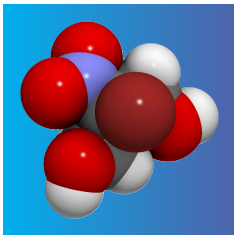
The purpose of this test was to evaluate the effect the RGF AOT unit has on mold/yeast bacteria (TPC). This test was performed utilizing a standard 2000 sq. ft. home and 3000 sq. ft. simulated home.

Tested by California Microbiology Center

Reduction %

Bacteria **99%** Mold **97- 98%** Yeast **90+%**





Chemical Compounds

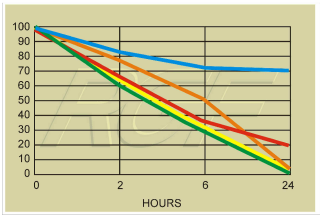
Gas Chromatograph/Mass Spectrometer test performed by Nelap Accredited Lab on airborne chemical compound reduction using RGF's AOT.

Hydrogen Sulfide - Rotten eggs
Methyl mercaptan - Rotten cabbage
Carbon Disulfide - Vegetable sulfide
Butyl Acetate - Sweet banana
Methyl Metharcyline - Plastic

Tested by GC/MS Nelap Accredited Independent Lab

Reduction %

Hydrogen Sulfide **80%** Methyl mercaptan **100%** Carbon Disulfide **30%** Butyl Acetate **100%** Methyl Metharcyline **100%**



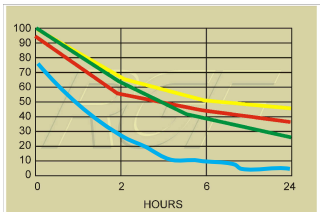
Odors

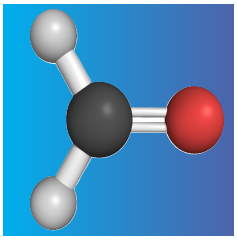
The purpose of this test was to evaluate to what effect the RGF's AOT unit has on cleaning chemicals, pet odors, smoke and perfume odors. This test was performed utilizing two 500 cubic foot test chambers and a ten-person odor panel. The qualitative assessments of the ten-person odor panel were then used as a means to determine the odor reduction.

Tested by C&W Engineering (Independent PE Firm)

Reduction %

Cleaning chemicals **55+%** Pet odors **72%** Perfume odors **63+%** Smoke odors **70%**

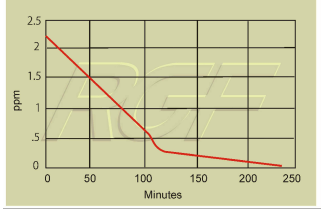




Formaldehyde

The purpose of this test was to evaluate the effect the RGF AOT unit has on formaldehyde.

Tests were conducted in a Class II Bio test chamber by Kansas State University



Electrical / Ozone / EMF

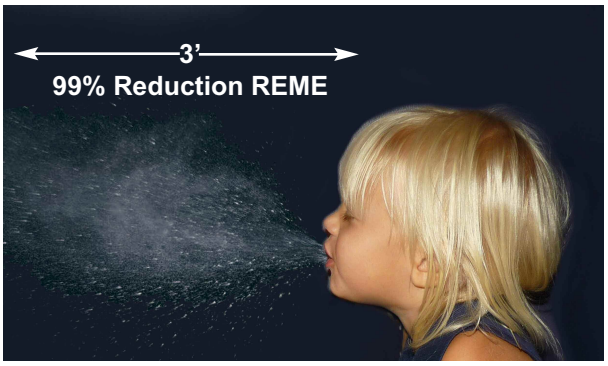
All Smell Fresh Arizona, LLC. AOP devices have been thouroghly tested for electrical safety, ozone / emf - Electro Magnetic Frequency and have passed Federal Safety Standards.

Tested by: TUV, ETL, UL, CSA, NEI China, RGF Labs. The Japanese Government, GSA, Electrical Power Research Institute.

Note: Many household appliances emit some ozone and emf in safe low levels such as and fluorescent lights, motors, computers, copy machines, refrigerators, blenders,electronic air filters, air conditioners, electric fans, microwave ovens, etc.

Sneeze Test

A testing protocol concept was used which included a "Sneeze Simulation Machine" and "Sneeze" chamber. A sneeze can travel at up to 100 mph, so we had to consider lung capacity, sneeze pressure, and liquid volume to properly simulate a human sneeze. This was accomplished and the test proceeded with outstanding results. An average of 88% reduction of microbials was achieved with PHI in a double blind test, at 3 feet from the sneeze source. This is clearly not a medically supervised test or protocol. However, from a practical point, it was definitely providing some kill at the source and will provide some level of protection. With some of our supplier's Advanced Oxidation devices, the same testing was performed and an average of 99% reduction of microbials was achieved in the same 3 foot distance.



Tested by: Kansas State University, inactivation 99% Simulated Sneeze Lab Test at three feet in a 250 cu ft Bio Test Chamber. An independent PE double blind study.

SAFETY

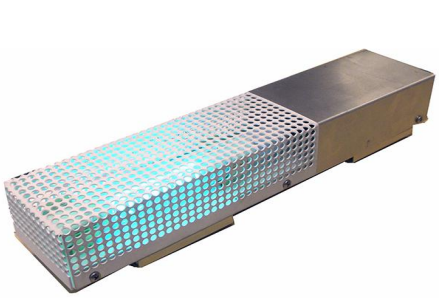
It is a normal reaction to question the long term safety of any product that is effective and uses new or "breakthrough" technology. This type of question has become common as our litigious society has taught us to question things that significantly outperform existing methods or products.

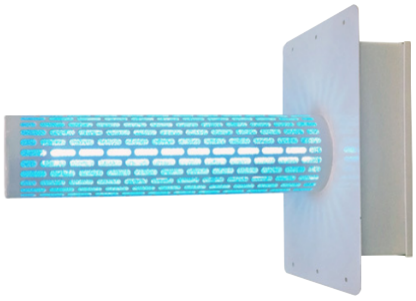
The *advanced oxidation technologies* that produced the results found on the pages of this report certainly fall into the category of breakthrough technology. This is evident by its outstanding test results across the entire range of microbes.

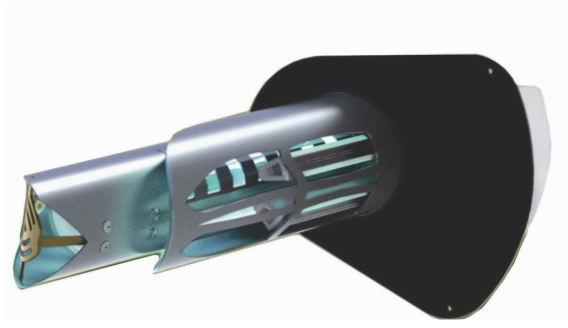
The breakthrough in the *advanced oxidation technologies* is not found in the final product (hydroperoxides) but rather in the method by which they are produced. The active ingredient created by these products is a group of oxidants known as Hydroperoxides. Hydroperoxides have been a common part of our environment for over 3.5 billion years. Hydroperoxides are created in our atmosphere whenever three components are present: unstable oxygen molecules, water vapor and energy (electro magnetic).

Hydroperoxides are very effective (as demonstrated by the test results in this book) at destroying harmful microbials. As oxidants, they do this by either destroying the microbe through a process known as cell lysing or by changing its molecular structure and rendering it harmless (which is the case in VOC's and odors). The amount of hydroperoxides required to accomplish this task in a conditioned space is well below the level that is constantly in our outside air. The advanced oxidation technology found in our product family has brought the oxidants found in the outside air into the conditioned space of your home, office, business, etc.

There is no known case of hydroperoxides ever creating a health risk. Considering we have been exposed to hydroperoxides in nature since the day man stepped on the planet, it is a reasonable assumption that hydroperoxides do not constitute a health risk. Over the past 20 plus years there are more than 1 million Advanced Oxidation products successfully used worldwide.







Disclaimer:

All the data above supplied and tests were performed by one of our suppliers (RGF) of Advanced Oxidation products with Advanced Oxidation Plasma of less than .02 ppm. They were conducted by independent accredited labs and university studies. They were funded and conducted by major clients to assure third party credibility. These products are not medical devices and no medical claims are made.

Smell Fresh Arizona, LLC.

PO Box 809, Phoenix, AZ 85001, U.S.A.

(623) 777-9883 • order@smellfresharizona.com • www.SmellFreshArizona.com

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Test No.

广州工业微生物检测中心
Guangzhou Testing Center of Industrial Microbiology
华南空气净化产品检测中心
South China Testing Center of Air Purification Products

检 测 报 告
TEST REPORT

广州工业微生物检测中心
Guangzhou Testing Center of Industrial Microbiology

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广州工业微生物检测中心

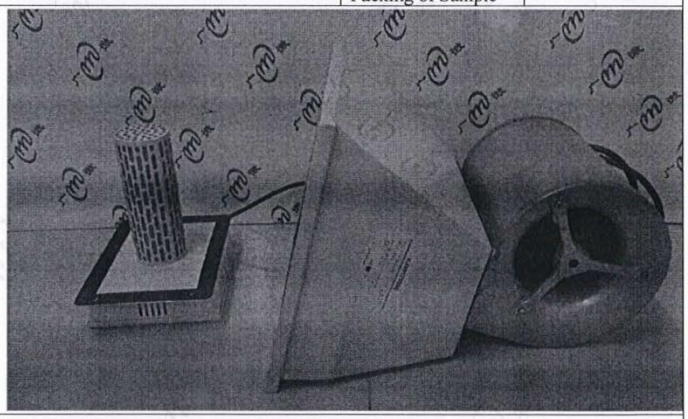
GUANGZHOU TESTING CENTER OF INDUSTRIAL MICROBIOLOGY

检 测 报 告

TEST REPORT

收样日期：2017 年 07 月 14 日
Date Received

检测日期：2017 年 07 月 15 日
Date Analyzed

样品名称 Name of Sample	VBK-GL-1000 UV Germicidal Air Purifier	样品来源 Source of Sample	Delivery Sample
委托单位 Applicant	V1 Environmental Technology Co., Ltd	委托人 Client	Pingtao Xie
生产单位 Manufacturer	V1 Environmental Technology Co., Ltd	商标 Brand	V1
型号规格 Type and Specification	VBK-GL-1000	样品数量 Quantity of Sample	1
生产日期 Date of Production	——	样品描述 State of Sample	
生产批号 Batch Number	——	样品包装 Packing of Sample	
样品图片 Sample Picture			
检验依据和方法 Standard and Methods	1. <Technical Standard For disinfection> 2002-2.1.3 Air disinfection effect evaluation test 2. GB21551.3-2010 Antibacterial and cleaning function for household and similar electrical appliances-Particular requirements of air cleaner		
检测项目 Items of Analysis	1.Simulated field test (staphylococcus albus 8032) 2.Field test (Natural bacteria in air) 3.Harmful Substances Release Amount (Ozone)		
备注 Remarks	——		

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Test Result				
Number of Sample	Test Item		Unit	Result
KJ20170865	Simulated field test (staphylococcus albus 8032)	1	%	99.99
		2		99.99
		3		99.99
	Field test (Natural bacteria in air)	1	%	93.13
		2		93.39
		3		93.82
	Harmful Substances Release Amount	Ozone	mg/m^3	<0.003

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广州工业微生物检测中心

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Method for Testing Air Disinfection(Simulated Field Test):

1. Test equipments
   1. Test microorganism: *Saphylococcus albus*
   2. Microbial aerosol generator: TK-3
   3. Culture media: NA
   4. Sampling equipment: six-stage sieve sampler
2. Test conditions
   1) The volume of the test chamber:10 m^3
   2) Environment temperature: (20～25) ℃
   3) Environment humidity: (50～70) % RH
3. Operation conditions of the machine
   Set the switch to position "The highest wind speed".
4. Test procedures
   1) Get a Bacteria slant culture (4~7 generation) which is incubated at 37 ℃ for 24 h, wash the culture from this slant with 10 mL NB, filter the liquid culture by aseptic cotton buds, and dilute this inoculums with NB as appropriate.
   2) The equipments are placed in the test chambers respectively, close the door, and open the HEPA filter. Simultaneously operate the environmental control devices until the experimental cabin temperature to be 20~25 ℃, relative humidity to be 50～70 %RH, Turn off the chamber environmental control system.
   3) Release microbial aerosol: turn on the microbial aerosol generator, release the microbial aerosol 15~20 min at 0.2 MPa, operate the ceiling mixing fan, then turn off the fan after 5 min, and let stand for 5 min.
   4) Original Bacteria aerosols collected by six-stage sieve sampler.
   5) The air cleaner are adjusted to the highest air cleaning mode setting for test (Test group), Bacteria aerosols (control group and test group) are collected at 30 min respectively.
   6) Choose 2 NA plates (the same batch) as the negative control, and culture them on the same condition with the samples.
   7) Run the test three times .
5. Computational formula

Natural decay rate $N_t(\%)=\frac{V_0-V_t}{V_0}\times 100$

Where: V_0 = Original Bacteria Count of Control group; V_t = Bacteria Count after Treatment of Control group .

Killing Rate $K_t(\%)=\frac{V_1\times(1-N_t)-V_2}{V_1\times(1-N_t)}\times 100$

Where: V_1 = Original Bacteria Count of test group; V_2 = Bacteria Count after Treatment of test group.

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Test results

Number of Sample	Test Time (min)	Test Bacteria	Test Number	Control group			Test group		Killing Rate K_t (%)
				Original Bacteria Count V_0 (cfu/m³)	Bacteria Count after Treatment V_t (cfu/m³)	Natural Decay Rate N_t (%)	Original Bacteria Count V_1 (cfu/m³)	Bacteria Count after Treatment V_2 (cfu/m³)	
KJ20170865-1	60	*Staphylococcus albus*	1	1.42×10^5	1.11×10^5	21.84	1.39×10^5	7	99.99
			2	1.45×10^5	1.09×10^5	24.83	1.43×10^5	7	99.99
			3	1.46×10^5	1.13×10^5	22.60	1.44×10^5	7	99.99

Test Conclusion: After 60mins test model VBK-GL-1000 air sterilizer in 10m3 chamber, the eliminated rate of Staphylococcus albus is ≥99.90% for 3 times.

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Test Method for Air Purifier on Airborne Bacteria (Field Test):

1. Test equipments:
   1) Culture media :NA
   2) Sampling equipment:six-stage sieve sampler
   3) The volume of the test chamber: $30m^3$
2. Operation conditions of the air purifier
   Set the switch to position"The highest wind speed".
3. Test Procedure
   1) The equipments are placed in the test chamber, close the door, and collect natural bacteria by six-stage sieve sampler, as the original bacteria count (positive control).
   2) The air purifier is adjusted to the highest air cleaning mode, turn on the unit for 60 min. The natural bacteria are collected by six-stage sieve sampler, as the bacteria count after treatment.
   3) In sampling, place the sampling equipment in the center of test chamber at the height 1.0 meter.
   4) Choose 2 NA plates (the same batch) as the negative control, and culture them on the same condition as the samples.
   5) The tests repeat three times, and calculate the killing rate respectively.
5. Killing Rate $K_t(\%)=\frac{V_0-V_t}{V_0}\times 100$

   where: V_0 = Original Bacteria Count; V_t = Bacteria Count after Treatment.

Test results

Number of Sample	Test Bacteria	Test time (min)	Test Number	Original Bacteria Count V_0 (cfu/m^3)	Bacteria Count after Treatment V_t (cfu/m^3)	Death rate K_t (%)
KJ20170865-1	Natural Bacteria in Air	60	1	1.47×10^4	1.01×10^3	93.13
			2	1.39×10^4	9.19×10^2	93.39
			3	1.52×10^4	9.40×10^2	93.82

Test Conclusion: After 60mins test model VBK-GL-1000 air sterilizer in 30m3 chamber, the eliminated rate of Natural Bacteria in Air is ≥90% for 3 times.

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Method for Testing Harmful Substances Release Amount:

1. Test Equipment
   Ozone Analyzer
2. Operation Conditions of the Machine
   Set the switch to position "The highest wind speed".
3. Test Procedures
   1) Put the test sample into a clean space.
   2) Test the background concentration.
   3) Turn on the test unit. Test the concentration values according to the standard requirements.

Test Results

Number of Sample	Items	Units	Results	Standard request (GB 21551.3-2010)
KJ20170865-1	Ozone	mg/m^3	<0.003	≤0.10

报告结束/End of report

编制：黄永良 Editor　审核：Checker　签发：Issuer　签发日期（公章）：2017.9.5 Date Reported

广州工业微生物检测中心 检验检测专用章

紫外线灯工作原理 Workding principle of UV lamp

紫外线杀菌灯和日光灯、节能灯发光原理一样，灯管内的汞原子被激发产生汞的特征谱线。低压汞蒸气主要产生 254nm 和 185nm 紫外线

日光灯、节能灯灯管采用的是普通玻璃，紫外线不能透出来，被荧光粉吸收后发出可见光；而杀菌灯灯管则用透紫外玻璃或石英玻璃生产。紫外线能穿过玻管壁透射出来。

Ultraviolet lamps produce 254nm & 185nm Uvrays due to low pressure mercury vapor,as fluorecent lamps and energy saving lamps do. The glass of fluorecent and energy saving lamps is regular,which can’ t radiate outside ,but absorablr to phorsphor then change to visual.Ultraviolet lamps use quartz glass or special glass that uv rays.

杀菌原理和特点 Principle and Characteristic for germicidal Function

254nm 波长的紫外线很易被生物体吸收，如图 1，作用于生物体的遗传物质 DNA，使 DNA 遭到破坏而导致细菌死亡。185nm 波长紫外线与空气作用可产生有强氧作用的臭氧，可有效地杀灭细菌。

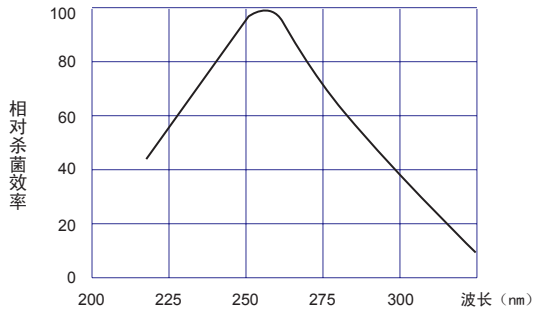
紫外线可集中很高的强度在短时间内杀灭细菌和病毒；如表 1 是紫外线对各种细菌和病毒杀灭效率的描述。紫外线杀菌属于纯物理消毒方法，无二次污染。

54nm UV rays is readily absorbable to cell,(see table 1),destroy genome DNA and kill bacteria ,so called Bactercide lamps.

Killbacteria and virus in a very short tima.

Please see chart 1.

Physical disinfection,no second time pollution.



（图1） 不同波长杀菌效率不同

杀灭对象 name		秒（S）	杀灭对象 name		秒（S）
细菌类 Bacteria	炭疽杆菌 Bacillus Anthracis	0.3	霉菌孢子 Mould Spores	黑曲霉 Aspergillus Niger	0.3-0.7
	破伤风杆菌 Clostridium Tetani	0.3		毛霉菌属 Mucor Mucedo	4.6
	痢疾杆菌 Dysentery bacilli	1.5		青霉菌属 Penicillium Roqueforti	0.9-3.0
	大肠杆菌 Escherichia Coli	0.4	水藻类 Algae	兰绿藻 Blue-Green Alage	10-40
	葡萄球菌属 Staphylococcus albus	1.3		线虫卵 Nematode Eggs	3.4
	结核杆菌 Micrococcus Candidus	0.4		绿藻 Green Algae	1.2
病毒 Virus	嗜菌胞病毒 Bacteiophage	0.2		原生动物类 Protozoa	4.0-6.7
	流感病毒 Influenza	0.3	鱼类病	白斑病	2.7
	脊髓灰质炎病毒 Poliovirus 1	0.8		感人性胰坏死病	4.0
	乙肝病毒 Hepatits B Virus	0.8		病菌性出血病	1.6

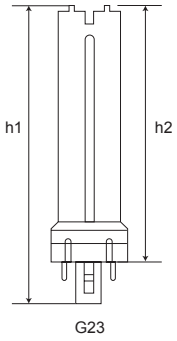
表 1 紫外线对常见细菌病毒的杀菌时间（假设辐射强度：30000μw/cm²）

如：辐射强度为 3000，要达到同样的杀菌效果，则时间要延长 10 陪，以此类推

Chart1 The Required Irradiation Time To Inactivate Microorganisms under 30,000μws/cm2 Dose of UV254nm

注：* 为 99.99% 杀灭所需时间。99.99% Lethal Dosage (Second)

单端 H 型紫外线杀菌灯 Single—ende H shape UV germicidal lamps



型号 Product code	管径 Diameter	灯长 Base face Length(mm)		功率 Wattage	管压 Lamp voltage	管流 Lamp current	1 米紫外线强度 UV intensity (Distance=1M)
	mm	h1 $^{+0}_{-2}$	h2	W	V	mA	Uw/cm^2
VBK-GL-1000 device			164	13	48—62	300	40

例：ZW13D12Y(W)-H164 表示：紫外线杀菌灯，13W, 单端 (D), 管径 12mm, 有臭氧 (Y) 无臭氧则应为 (W), H 管 (H), 长度 164mm。

产品特点：

可内置 110 伏或 220 伏跳泡，抗干扰电容；
使用电子镇流器时，不加跳泡，内置启动电容；
尺寸更短，安装方便。

features：

110V or 220V starter and capacitor inside.
Only capacitor needed by using electronic ballast；
More compact ，easy to install.

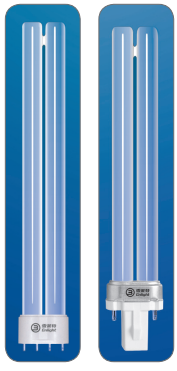
应用范围：

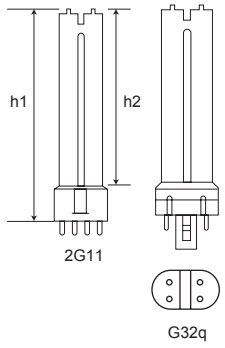
消毒、杀菌、除臭、净化仪器与设备中；医疗消毒机，光催化空气净化器，水族消毒等。

applications：

Medical disinfection and air clean ，deodorization and bactericide aquarium UV sterilizers.

单型 HB 型紫外线杀菌灯 Single—ende HB shape UV germicidal lamps





型号 Product code	管径 Diameter	灯长 Base face Length(mm)		功率 Wattage	管压 Lamp voltage	管流 Lamp current	1 米紫外线强度 UV intensity (Distance=1M)
	mm	h1 $^{+0}_{-2}$	h2	W	V	mA	Uw/cm^2
VBK-GL-2000 device	17	217	195	18	52—64	375	57
VBK-GL-3000 device	17	317	295	24	77—97	345	94
VBK-GL-4000 device	17	411	379	36	98—118	435	147

例：ZW55D17Y(W)-H533 表示：紫外线杀菌灯，55W ,单端 (D), 管径 17mm, 有臭氧 (Y) 无臭氧则应为 (W), H 管 (H), 长度 533mm。

备注：40W、50W 只可配电子镇流器使用

产品特点：

可采用 2G11 或 G32q 灯头 ,G32q 灯头可内置跳泡和电容 ,2G11 灯头为外启动;
可生产大功率灯，产生高臭氧，对大范围空间消毒等；
可配置相应的反光灯盘或反罩光；

features：

Base: 2G11 or G32q，the latter can be put starter and capacitor inside.
High Wattages can be made, high density ozone disinfects big areas.
Reflector or reflector plate matched.

应用范围：

应用于医院手术室、病房的消毒、杀菌、除臭、净化仪器与设备中；
医疗用空气消毒机，光催化空气净化器，水族消毒等。

applications：

Medical disinfection and air clean ，deodorization and bactericide aquarium UV sterilizers.

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Inactivation of Viruses on Surfaces by Ultraviolet Germicidal Irradiation

Chun-Chieh Tseng and Chih-Shan Li

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Abstract

In many outbreaks caused by viruses, the transmission of the agents can occur through contaminated environmental surfaces. Because of the increasing incidence of viral infections, there is a need to evaluate novel engineering control methods for inactivation of viruses on surfaces. Ultraviolet germicidal irradiation (UVGI) is considered a promising method to inactivate viruses. This study evaluated UVGI effectiveness for viruses on the surface of gelatin-based medium in a UV exposure chamber. The effects of UV dose, viral nucleic acid type (single-stranded RNA, ssRNA; single-stranded DNA, ssDNA; double-stranded RNA, dsRNA; and double-stranded DNA, dsDNA), and relative humidity on the virus survival fraction were investigated. For 90% viral reduction, the UV dose was 1.32 to 3.20 mJ/cm^2 for ssRNA, 2.50 to to 4.47 mJ/cm^2 for ssDNA, 3.80 to 5.36 mJ/cm^2 for dsRNA, and 7.70 to 8.13 mJ/cm^2 for dsDNA. For all four tested viruses, the UV dose for 99% viral reduction was 2 times higher than those for 90% viral reduction. Viruses on a surface with single-stranded nucleic acid (ssRNA and ssDNA) were more susceptible to UV inactivation than viruses with double-stranded nucleic acid (dsRNA and dsDNA). For the same viral reduction, the UV dose at 85% relative humidity (RH) was higher than that at 55% RH. In summary, results showed that UVGI was an effective method for inactivation of viruses on surfaces.

Keywords: bacteriophage, inactivation, surfaces, UVGI, virus

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INTRODUCTION

Viruses are obligate parasites that cannot multiply or propagate outside specific host cells. In the environment, surfaces become contaminated with viruses through contact with infectious body fluids or the settling of airborne viral particles. For surfaces to serve as sources of viral disease, the involved virus must be able to survive in association with the surface until it encounters a susceptible host. There have been a number of viral outbreaks related to surface-related transmission, such as hepatitis virus,[1, 2] rotavirus,[3, 4] enterovirus,[5, 6] and severe acute respiratory syndrome coronavirus (SARS CoV).[7, 8]

Recently, enteric viruses and SARS CoV have emerged as major public health issues due to their ability to spread through close person-to-person contact and their transmission by droplets generated by an infected person. Enterovirus and SARS CoV could also be spread when a person touches a surface contaminated with infected droplets and then touches their nose, mouth, or eyes. In Taiwan, enterovirus 71 caused 78 deaths in a large outbreak in 1998, and reoccurred in recent years.[5, 6] The outbreak was highly related to contact transmission and resulted in the widespread of hand-foot-and-mouth disease among exposed population. For SARS CoV, the outbreak occurred in Taiwan with 73 deaths in 2003. Some evidence reveled that polymerase chain reaction (PCR) positive swab samples were recovered from frequently touched surfaces in rooms occupied by SARS patients and in nurse stations used by staff.[9, 10] These observations led to speculation that a possible route of SARS CoV transmission was contact with environmental surfaces.

There are many control techniques that could reduce risk from viral infection on surfaces, including heating sterilization,[11] ultraviolet germicidal irradiation (UVGI),[12] and chemical disinfectants.[13] However, many surface materials cannot be heat sterilized and might be damaged by chemical disinfection.[14]

In contrast with most disinfectants, UVGI has been well recognized as an effective method for inactivating microorganisms.[12, 15–18] The mechanisms of UVGI on microorganisms are uniquely vulnerable to light at wavelengths at or near 253.7 nm because the maximum absorption wavelength of a DNA molecule is 260 nm.[19] After UV irradiation, the DNA sequence of microorganisms can form pyrimidine dimers, which can interfere with DNA duplication, as well as lead to destruction of nucleic acids and render the viruses noninfectious.[20]

In addition, UVGI effectiveness for microorganisms inactivation was related to irradiation level, duration of irradiation, and relative humidity (RH).[21–23] Until now, the UVGI virus-related investigations evaluated only viruses in water[24–26] and air;[27] these studies found that UVGI would effectively inactivate viruses in water and in their airborne phases. Moreover, the type of viral nucleic acid, host cell repair mechanisms, and capsid structure of virus played an important role in virus inactivation.[26] However, there is little information available on the effectiveness of UVGI for inactivation of viruses on surfaces.

The purpose of this study was to determine the effectiveness of UVGI for virus inactivation on surfaces. For selection of virus target, it is believed that radiation would restructure the nucleic

acid of the microorganisms and destroy its replication ability; therefore, the type of the viral nucleic acid may play a critical role on virus inactivation by UVGI.

According to the types of the nucleic acids, viruses can be divided into four groups, including single-stranded RNA (ssRNA), single-stranded DNA (ssDNA), double-stranded RNA (dsRNA), and double-stranded DNA (dsDNA). In addition, bacteriophages are more resistant to UVGI than other pathogenic viruses; therefore, they are considered as suitable indicators. The bacteriophages used in this study have been used as indicators of poliovirus, enterovirus, enveloped viruses, and human immunodeficiency virus.[28–30] Consequently, this study evaluated the effects of UV dose, different nucleic acid type of virus (four different bacteriophages with ssDNA, ssRNA, dsDNA, and dsNA), and RH (55% and 85%) on virus survival fraction after UVGI exposure.

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METHODS

Test Viruses

In medical and environmental virology applications, bacteriophages have widely served as suitable surrogates for mammalian viruses.[28–30] In this study, the tested viruses were four different bacteriophages: ssRNA (MS2, American Type Culture Collection, ATCC 15597-B1), ssDNA (phi X174, ATCC 13706-B1), dsRNA (phi 6 with envelope lipid, ATCC 21781-B1), and dsDNA (T7, ATCC 11303-B1). The host bacteria were *Escherichia coli* F-amp (ATCC 15597) for MS2, *E. coli* CN-13 (ATCC 13706) for phi X174, *E. coli* 11303 (ATCC 11303) for T7, as well as *Pseudomonas syringae* (ATCC 21781) for phi 6.

In the current study, a high titer stock of bacteriophages (10^9–10^{10} PFU/ml, where PFU is plaque forming units) was prepared via plate lysis and elution.[31] Moreover, the plaque assay[32] for determining virus infectivity and phage cultivation methods were all followed from the ATCC product information sheet. To allow the phage to attach to the host, the bacteriophages were mixed with their own respective host.

First, 5 mL molten top agarose (containing only 0.7% agarose) was added to a sterile tube of infected bacteria. The medium for MS2, phi X174, T7, and phi 6 phage cultivation included Luria-Bertani agar (244520; Difco Laboratories, Detroit, Mich.), nutrient agar (213000; Difco) with 0.5 NaCl, trypticase soy agar (236950; Difco), and NBY agar (containing nutrient broth, yeast extract, K_2HPO_4, KH_2PO_4, and $MgSO_4 \cdot 7H_2O$), respectively. Then the contents of the tube were mixed by gentle tapping for 5 sec and poured onto the center of a labeled agar plate.

Finally, the plate was incubated for 24 hr either at 37°C for coliphages or at 26°C for phi 6. After cultivation, 5 mL SM buffer (containing NaCl, $MgSO_4 \cdot 7H_2O$, Tris, and gelatin) was pipetted onto a plate that showed confluent lysis. Then the plate was slowly rocked by a mechanical shaker (model OS701, TKS Orbital Shaker; Taipei, Taiwan), for 40 min and the buffer was transferred to a tube for centrifugation in a Kubota centrifuge (Kubota Corporation, Tokyo, Japan) at 4000 × g for 10 min. After the supernatant was removed, the remaining phage stock

was kept at −80°C. From our preliminary results (data not shown), virus infectivity could be maintained for 24 hr at 4°C. For UVGI experiments, the virus titers were determined by plaque assay, and the virus suspension was stored at 4°C within 24 hr.

Surface Test System

Gelatin-Based Medium

From an earlier study,[12] there is little data available for virus inactivation by UVGI on surfaces. Another study[33] showed that different kinds of surface compositions to which viruses were adsorbed may cause viruses to lose their infectivity because of desiccation. Therefore, the stability of virus infectivity on the evaluated surface is very important. In the current study, a gelatin-based medium was used for the tested surface because it offered a more ideal growth medium for the viruses, preserving their infectivity. From our preliminary tests (data not shown), virus infectivity remained the same at least for 1 hr at RH 55% and 85% (with coefficient of concentration variation below 20%).

The gelatin-based medium was composed of LB (Luria-Bertani) broth with 7% gelatin. After sterilization by autoclave (121°C, 15 min) the medium solidified at 4°C. Then, a diluted culture of virus stock solution (10^9 PFU/mL, 0.1 mL) was spread on the surface of the gelatin medium and naturally air dried (20 min) in a laminar flow hood to prevent contamination. On each plate, the virus concentration was 10^8 PFU/mL. After UVGI exposure, the gelatin-based medium was directly liquefied at 37°C in a incubator (model LT1601; TKS Technology, Taiwan) for further quantification without elution procedure. Finally, the viral particles in the liquid phase of the medium were subjected to plaque assay for coliphages at 37°C and for phi 6 at 26°C. Experiments were performed at least in triplicate for each set of conditions with different UV dose.

All plates (both UV-exposed and UV-unexposed) were incubated for 24 hr. The virus survival fraction was calculated as the ratio of the number of plaques forming on the UVGI-exposed plates compared with the number of plaques on the UVGI-unexposed control plates. All experiments were conducted in darkness to prevent visible light effects.[34] The test system was located in a chemical hood so that the exhausted gas was vented outside.

RH Regulation Unit

A humidified gas stream was generated by passing pure compressed air through a humidity saturator (Figure 1). Sterile deionized water was used in the saturator. Water vapor content (i.e., RH) in the gas stream was adjusted by changing the flow rate ratio of the humidified gas stream to a dry gas stream and was finally measured using a hygrometer (Sekunden-Hygrometer 601; Testo, Lenzkirch, Germany) placed in the UV exposure chamber. The gas flow rates in the chamber was 10 L/min. For evaluating the effect of RH, the humidified gas stream was heated by adding a dry gas stream to reach a medium (RH 55%) or very humid condition (85%) at 25–28°C.

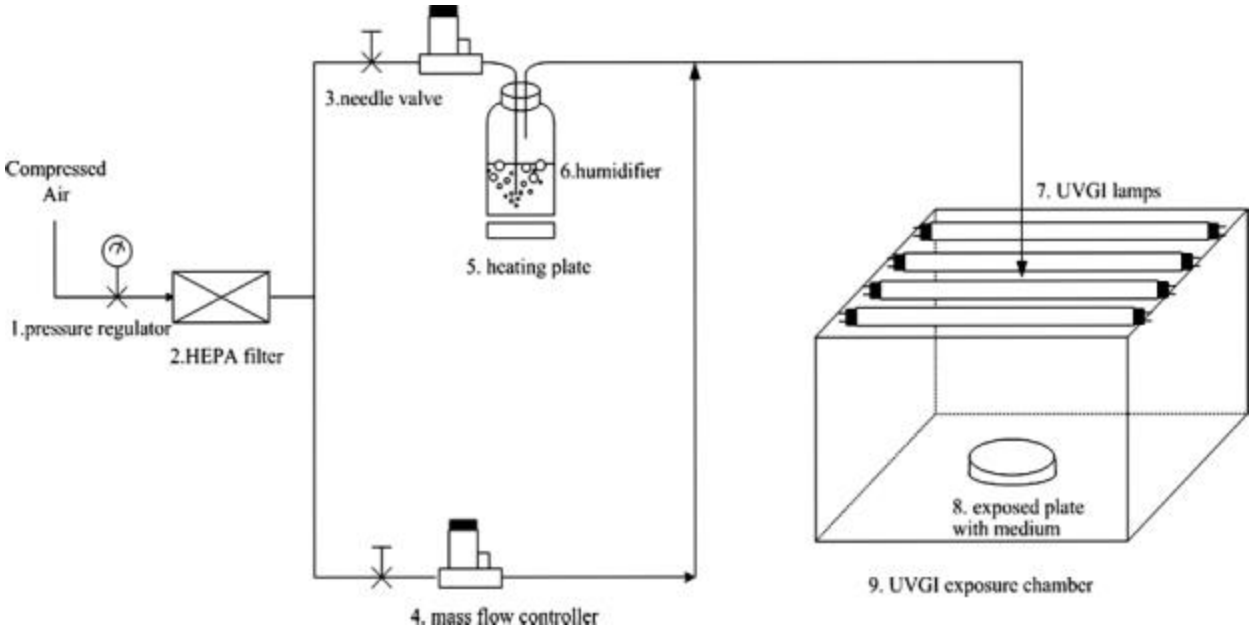

FIGURE 1.

Schematic of experimental apparatus used for evaluation of UVGI effectiveness for inactivation of viruses on the surface.

UV Exposure Unit

The UV exposure chamber was approximately 0.02 m^3 in volume (0.27 m × 0.30 m × 0.3 m). The exposed samples were irradiated with four 8W UV-C lamps (germicidal lamp, TUV 8W/G8 T5; Philips Electronic Instruments, Eindhoven, The Netherlands) with a radiation peak at 253.7 nm for germicidal action. The lamps were placed 30.5 cm above the surface of the medium and were wrapped in a layer of cellophane to attenuate original irradiation magnitude. The intensity of UVGI on the surface of the medium was measured using an UV-radiometer (P-97503-00; Cole-Parmer, Vernon Hills, USA) with a 254-nm sensor.

In a preliminary study (data not shown), UV intensity in the range of 60 to 240 $\mu W/cm^2$ was used, and the exposure time varied from 3 sec to 6 min. Because the UV dose is the product of the UV intensity and UV exposure time, the evaluated UV doses were in the range of 0.18 to 86.4 mJ/cm^2. In the current study, experiments were performed at least in triplicate for each set of conditions for UV intensity (120 $\mu W/cm^2$), exposure time (5, 15, 35, 85, 165, 255 sec), RH (55% and 85%), and tested virus (MS2, phi X174, T7, and phi 6).

Survival Fraction of Viruses vs. UVGI Exposure

The total dose to which a virus on a surface was exposed was defined as the product of the UVGI intensity *I* on the viruses and the exposure time *t*. The survival fraction is the ratio that represents the virus concentration after UVGI exposure. Microorganisms susceptibility factor (K-value) was derived from the exponential decay model presented in following equation:

$$\frac{N_{uv}}{N_0} = e^{-KIt}$$

(1)

where

- N_{uv} = concentration of virus surviving after exposure to UVGI (PFU/mL)
- N_0 = concentration of virus unexposed to UVGI (PFU/mL)
- I = UV intensity (μW/cm^2)
- t = UV exposure time (sec)
- K = microorganism susceptibility factor (cm^2/mJ)

Statistical Analysis

The log survival data vs. UV dose for each experiment was used to perform regression analysis on the data for each virus. R^2 values were obtained by regression analysis. Generation of regression lines and prediction of the doses required for 90% and 99% viral reduction were accomplished by including data points from all experiments for each tested virus. Comparisons of survival fraction among the viruses were performed using t test to evaluate statistically significant differences ($p < 0.05$).

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RESULTS

Results of the survival fraction of four bacteriophages at two RH conditions are presented in Figures Figures22 and and3.3. The survival fractions of all four viruses were found to be inversely related to UV dose. For 90% viral reduction, the ssRNA virus (MS2) required an extremely low UV dose (1.32 to 3.20 mJ/cm^2), ssDNA virus (phi X174) was more resistant than MS2 and required a higher UV dose (2.50 to 4.47 mJ/cm^2), dsRNA (phi 6) required a relatively higher dose (3.80 to 5.36 mJ/cm^2), and dsDNA virus (T7) required a high dose (7.70 to 8.13 mJ/cm^2).

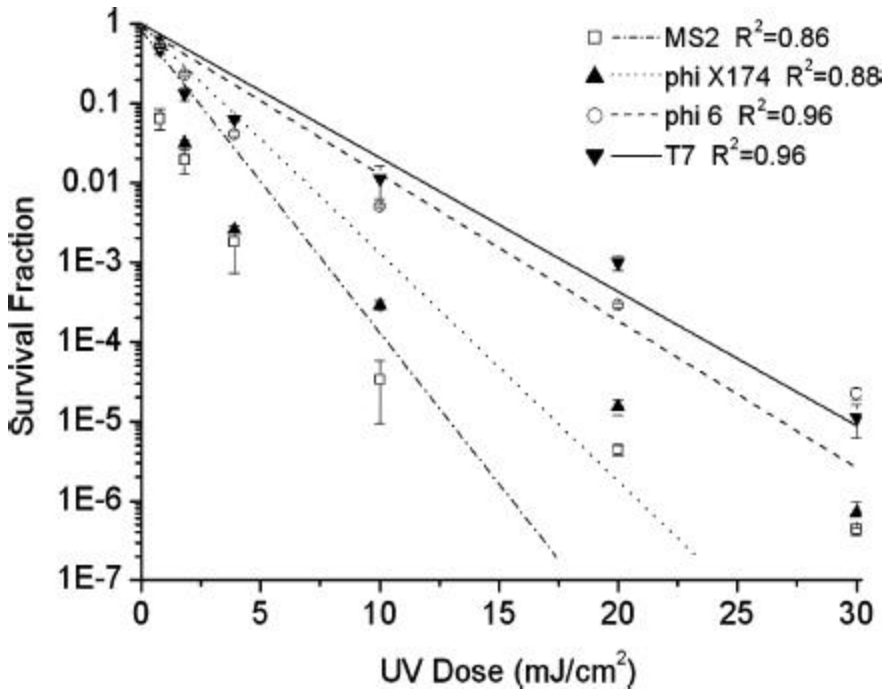

FIGURE 2.

Survival fraction of surface viruses (MS2, phi X174, phi 6, and T7) exposed to UVGI at RH 55%. Error bars represent one standard deviation of the mean of at least three trials.

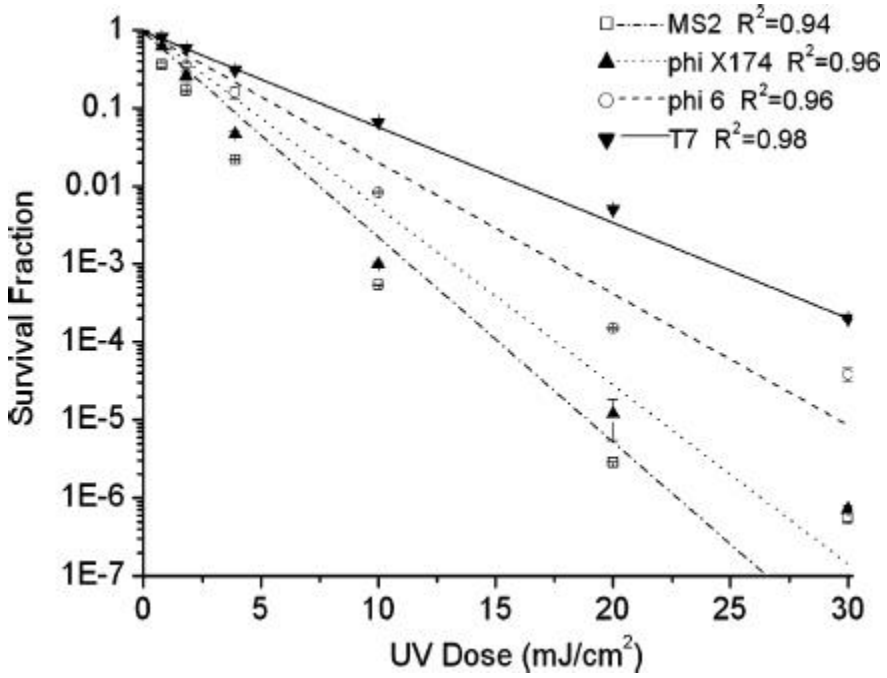

FIGURE 3.

Survival fraction of surface viruses (MS2, phi X174, phi 6, and T7) exposed to UVGI at RH 85%. Error bars represent one standard deviation of the mean of at least three trials.

These results clearly indicate that dsRNA and dsDNA viruses are more resistant to UVGI than those of ssRNA and ssDNA viruses (UV doses for dsRNA and dsDNA was approximately 3 times higher than those for ssRNA and ssDNA, $p < 0.05$). For 99% viral reduction, the UV dose for MS2 ranged from 2.51 to 6.50 mJ/cm^2, for phi X174 from 5.04 to 8.34 mJ/cm^2, for ph 6 from 7.75 to 10.57 mJ/cm^2, and for T7 from 15.54 to 16.20 mJ/cm^2. These results indicate that the dose for viral reduction of dsRNA and dsDNA viruses on a surface is approximately 3 times higher than those of ssRNA and ssDNA viruses ($p < 0.05$).

Based on exponential decay model, the microorganism susceptibility factors, *K* value, varied widely. K values of ssRNA/DNA viruses were higher than those of dsRNA/DNA viruses. This could be because dsRNA/DNA viruses are more resistant to UV irradiation than ssRNA/DNA viruses. For the four types of viruses, *K* values (0.27–0.64 cm^2/mJ) at 85% RH were lower than those (0.3–0.83 cm^2/mJ) at 55% RH (Figures (Figures22 and and3),3), which demonstrates that a higher UV dose is required to inactivate viruses at higher RH ($p < 0.05$). This finding was in agreement with our previous bacterial and fungal findings.[,22]

These results could be explained by possible water sorption onto viruses, which provides protection against UV-induced DNA or RNA damage at higher RH. Moreover, the RH effects on UVGI effectiveness were also related to the type of virus nucleic acid. The RH effects on UVGI inactivation of ssRNA and ssDNA viruses on surfaces were greater than those of dsDNA and dsRNA.

The effectiveness of UVGI for viruses inactivation on surfaces was fitted well with an exponential decay model. Moreover, observations are in agreement with the Bunsen-Roscoe reciprocity law[,35] that states if a photobiologic effect depends purely on photochemical events, the biologic effect of a UV exposure depends on the product of the irradiance and exposure time. In summary, the UVGI effects for virus inactivation on surface depended on UV dose and percent RH. For all nucleic acid types of virus, the survival fraction decreased exponentially at higher UV dose.

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DISCUSSION

In air, an airborne virus with dsDNA (adenovirus) was less susceptible to UVGI than viruses with ssRNA (Coxsackie B1 virus, Influenza A virus, Sindbis virus and Vaccinia virus).[,27] In suspension, MS2 has higher resistance to UVGI than other ssRNA viruses (feline calicivirus, Ecovirus, Coxsackie virus, and poliovirus) or dsDNA virus (PRD1).[,24–26] MS2 was more susceptible to UVGI than those of phi 6 and T7 because the complex nucleic acids (doubled strained genomes) of both phi 6 and T7 could enable these two phages to use the host enzymes to repair damages. In addition, bacteriophages are more resistant to UVGI than other pathogenic

viruses in the environment. Therefore, these viruses may be less susceptible to UVGI inactivation than the bacteriophages used in this study.

In previous investigations,[16–17] solid media that included beef extract agar, beef-infusion blood agar, and malt extract agar were widely used for inactivation of microorganisms on surfaces by UVGI. Regarding inactivation of other microorganisms on surfaces, the UV doses for 90% viral reduction were similar to those for *E. coli, Serratia marcescens, Staphylococcus haemolyticus, Salmonella typhi, Streptococcus viridans, Staphylococcus albus, Shigella paradysenteriae*, and yeast (1.7–7.4 mJ/cm^2) but much lower than for *Bacillus subtilis* (19 mJ/cm^2) and *Penicillium citrinum* (22 mJ/cm^2).

These findings revealed that virus susceptibility to UVGI was similar to that of nonsporultating species, such as fragile bacteria and yeasts, but is higher than that for endospore-forming bacteria and fungal spores. Moreover, the susceptibility of microorganisms to UV irradiation was highly related to the presence or absence of a cell wall, cell wall thickness, and the type of nucleic acid.

In comparison with airborne evaluation,[36] it was demonstrated that UV lethal radiation doses required for airborne viruses were lower than those for viruses on surfaces. Furthermore, the ratio of the 90% viral reduction dose for virus on surfaces to airborne viruses ranged from 3.9 to 7.6 for MS2, from 5.6 to 9.0 for phi X174, from 5.7 to 6.2 for phi 6, and from 6.8 to 8.5 for T7. This may be explained by the fact that viruses can form aggregation on surfaces. When compared with studies where UVGI effectiveness was investigated on viruses in suspension, much higher UV doses were needed for 90% inactivation of MS2 virus (12–24 mJ/cm^2) than on a surface (1.32 to 3.2 mJ/cm^2).[25, 26] Viruses may be less susceptible to UVGI when associated with water.

This study used gelatin-based medium as the test surface for evaluating the susceptibility of viruses to UVGI. Gelatin is a protein source and solidifying agent for use in preparing microbiological culture medium. The smooth surface of this medium is suitable for viruses to preserve their infectivity. In UVGI applications, the UVGI susceptibility of viruses may be changed because of different kinds of surface compositions to which viruses are adsorbed. Viruses may be more susceptible to UVGI on the growth media because of desiccation.

When considering UVGI application to inactivate viruses on the surface, care needs to be taken, since it is known that microorganisms' growth could occur in crevices, and UVGI cannot completely penetrate these shadowed areas. Moreover, UVGI could damage or discolor surfaces and cause possible health effects, such as erythema of the skin and photoleratitis; therefore, humans, plants, and animals should be removed from the area when UVGI is applied. In summary, the effectiveness of UVGI for viral reduction on surfaces may be associated with the type of virus nucleic acid. Viruses with dsRNA or dsDNA may be less susceptible to UVGI inactivation. At high RH, a higher UV dose was required to inactivate virus on surfaces.

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CONCLUSIONS

The effects of UV dose, type of virus nucleic acid, and RH on the effectiveness of UVGI to inactivate surface viruses were evaluated in a UV exposure chamber. For virus inactivation on the surface, the effectiveness of UVGI strongly depended on a type of virus nucleic acid. Viruses with dsRNA or dsDNA could be less susceptible to UVGI inactivation. For 90% surface virus inactivation, the UV dose for dsRNA and dsDNA viruses was approximately 2 to 3 times higher than ssRNA and ssDNA viruses, respectively. The susceptibility factor for the viruses was higher at 55% RH than at 85% RH possibly because when RH increases, water sorption onto the virus surface might provide protection against UV-induced DNA or RNA damage.

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Ionizing air affects influenza virus infectivity and prevents airborne-transmission

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Abstract

By the use of a modified ionizer device we describe effective prevention of airborne transmitted influenza A (strain Panama 99) virus infection between animals and inactivation of virus (>97%). Active ionizer prevented 100% (4/4) of guinea pigs from infection. Moreover, the device effectively captured airborne transmitted calicivirus, rotavirus and influenza virus, with recovery rates up to 21% after 40 min in a 19 m^3 room. The ionizer generates negative ions, rendering airborne particles/aerosol droplets negatively charged and electrostatically attracts them to a positively charged collector plate. Trapped viruses are then identified by reverse transcription quantitative real-time PCR. The device enables unique possibilities for rapid and simple removal of virus from air and offers possibilities to simultaneously identify and prevent airborne transmission of viruses.

There is an urgent need for simple, portable and sensitive devices to collect, eliminate and identify viruses from air, to rapidly detect and prevent outbreaks and spread of infectious diseases[1]. Each year, infectious diseases cause millions of deaths around the world and many of the most common infectious pathogens are spread by droplets or aerosols caused by cough, sneeze, vomiting etc.[2,3,4,5]. Knowledge of aerosol transmission mechanisms are limited for most pathogens, although spread by air is an important transmission route for many pathogens including viruses[6].

Today no simple validated technology exists which can rapidly and easily collect viruses from air and identify them. The problem is not the analyzing technique, since molecular biological methods such as real-time PCR enable a sensitive detection system of most pathogens[7,8,9]. The difficulty is to develop an effective sampling method to rapidly collect small airborne particles including viruses from large volumes of air. Furthermore, the sampling method should be robust

with easy handling to enable a wide distribution and application in many types of environment. At present, the most commonly used techniques aimed to collect pathogens from air are airflow and liquid models[10,11,12,13,14,15]. These systems are complex, and their efficiency has not been thoroughly evaluated.

Spread of infectious diseases in hospitals can be most significant[16,17,18]. In many situations there is a need for a pathogen- and particle-free environment, e.g. in operation wards, environments for immunosuppressed patients as well as for patients with serious allergies. This makes it desirable to have a method not only for collection and identification[19], but also for eliminating virus and other pathogens from air[20]. Ozone gas has been shown to inactivate norovirus and may be used in empty rooms to decontaminate surfaces, however in rooms with patients ozone should not been used due to its toxicity[21]. Generation of negative ions has previously been shown to reduce transmission of Newcastle disease virus[22,23] and several kind of bacteria[24,25] in animal experimental set-ups.

The ionizing device used in this study operates at 12 V and generates negative ionizations in an electric field, which collide with and charge the aerosol particles. Those are then captured by a positively charged collector plate. For safety reasons, the collector plate has a very low current, less than 80μA, however the ionizer accelerates a voltage of more than 200,000 eV, which enables high production of several billion electrons per second. Moreover, this device does not produce detectable levels of ozone and can thus be safely used in all environments.

This technique is known to effectively collect and eliminate cat-allergens from air[26]. Aerosolized rotavirus, calicivirus and influenza virus particles exposed to the ionizing device were attracted to the collector plate and subsequently identified by electron microscopy and reverse transcription quantitative real-time PCR techniques. Most importantly, we demonstrate that this technology can be used to prevent airborne-transmitted influenza virus infections.

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Results

Visualization and efficiency of aerosol sampling as determined by electron microscopy

To develop and validate the ionizing technique for collection and identification of viral pathogens, we used several viruses of clinical importance; calicivirus, rotavirus and influenza virus (H3N2, strain Salomon Island) as well as latex particles. Canine calicivirus (CaCV, strain 48) was used as a surrogate[27] for human norovirus, the aetiological agent behind the “winter vomiting disease”, causing outbreaks of great clinical and economic importance[28]. Rhesus rotavirus was used as a surrogate marker for human rotavirus[29].

The device (Fig. 1a) consists of a small portable 12 volt operated ionizer, with a collector plate of positive charge attached to the ionizer, attracting negative particles from the air by electrostatic attraction. To determine optimal time collection parameters, latex particles with sizes ranging from <1 to >10 μm were nebulized into a room of 19 m^3. Testing revealed that 40–

60 min was required to eliminate >90% of free latex particles in the air as determined by real-time particle counting (PortaCount Plus). The particle counter can detect particles with size greater than 0.02 μM. Visualization by scanning electron microscopy (SEM) on grids from active- and inactive ionizer collector plates showed that accumulation of latex particles was dramatically enhanced on active ionizer collector plates compared to the inactive (Fig. 1b,c). Next, high numbers of rotavirus and formalin-inactivated influenza virus were aerosolized under the same conditions. While, after 40 min the inactive collector plates contained few (<5) rotavirus and influenza virus, the active collector contained >50 virus particles, as determined by transmission electron microscopy (TEM), (Fig. 1d,e).

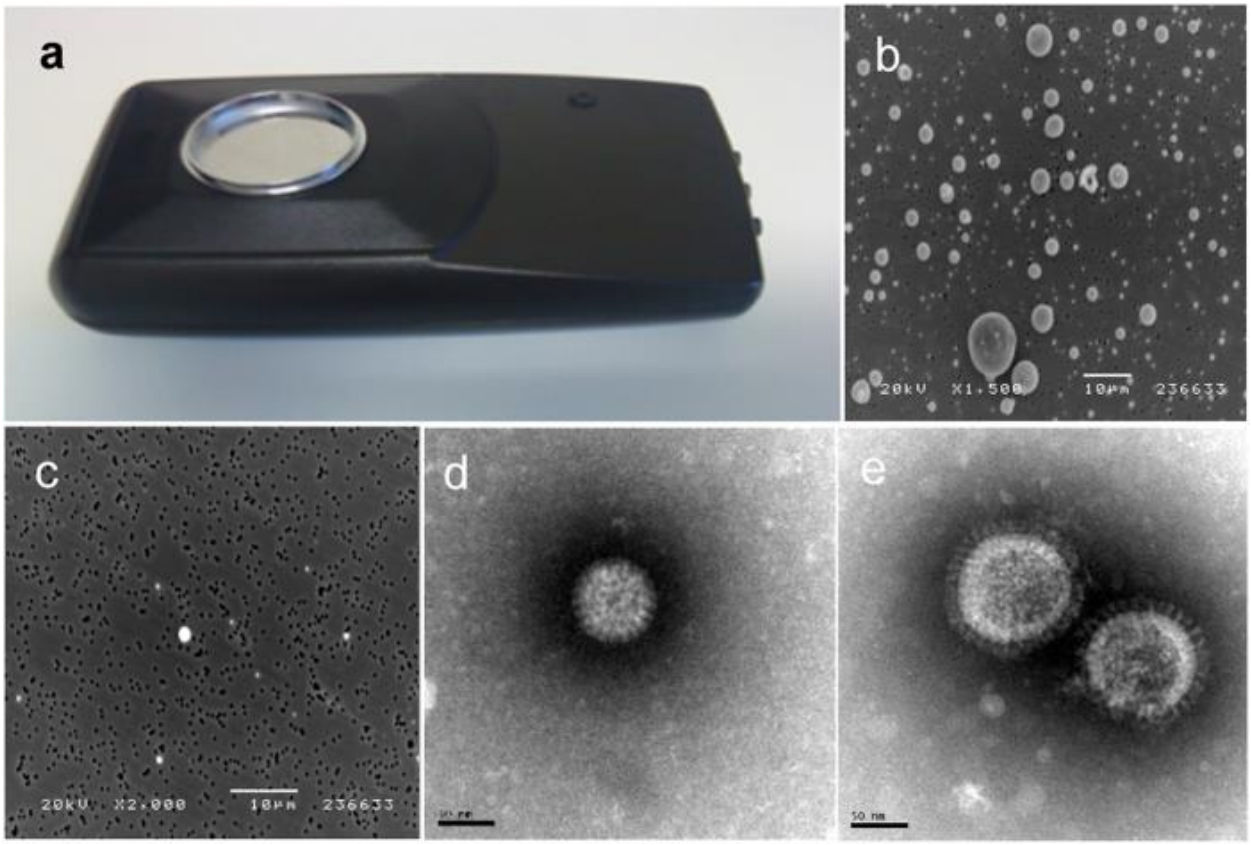

Figure 1

Airpoint ionizer with collector plate (size 13 × 35 cm) (**a**). The ionizing device was developed based of the Ion-Flow Ionizing Technology from LightAir AB, Solna, Sweden and was modified by installing a plastic-cup with a conductive surface of 47 mm in diameter, with positive charge, as the collector plate; Aerosolized and trapped latex particles (>1 to <10 μm) on active (**b**) and inactive (**c**) ionizer, (bar = 10 μM); Rotavirus (**d**); and influenza virus (H1N1; strain Salomon Island) (**e**) trapped on active ionizer, (Bar = 50 nm).

Ionizing air and electrostatic attraction collects aerosol-distributed viruses as determined by RT-qPCR

We next determined the capacity of RT-qPCR technology to quantitate the capacity of the ionizer technique to collect and concentrate viruses. Three independent experiments with each of the three viruses were carried out using the same virus concentrations in each experiment (Fig. 2a–c). Although several steps are involved from collection to detection the system was robust as to reproducibility. The RT-qPCR data shows that the active collector is concentrating and collecting virus 1500–3000 times more efficient as compared to the inactive collector (Table 1). When different dilutions of virus was used for aerosol production the proportion of aerosolized virus collected on the active collector was normally in the range of 0.1–0.6% for CaCV, rotavirus and influenza virus. A reproducible finding with regard to CaCV was a significant increase in relative recovery at the lowest concentrations increasing to 10–20% of the total amount of virus aerosolized (Table 1).

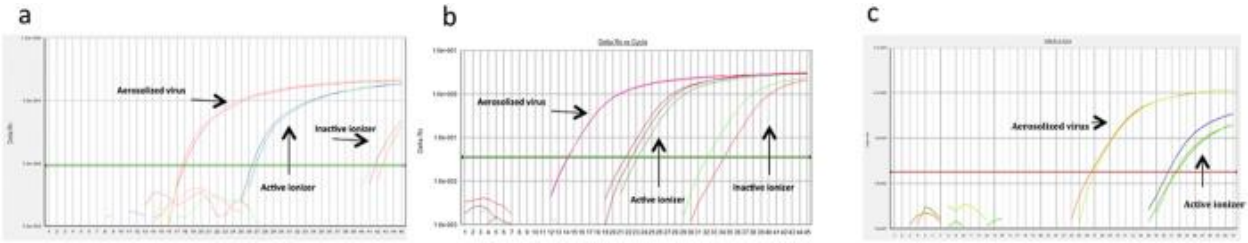

Figure 2

Real-time PCR on trapped rotavirus (**a**), calicivirus (**b**) and influenza virus (H1N1; strain Salomon Island) (**c**). Note that no influenza virus was detected on the inactive ionizer.

Table 1

Collection efficiency of aerosolized CaCV, rhesus rotavirus (RRV) and Influenza A virus in various concentrations as determined by RT-q PCR.

Amount virus aerosolized (genes/PCR reaction)	Amount virus on collector ON (genes/PCR reaction) ±SE	Amount virus on collector OFF (genes/PCR reaction)	Recovery (%) ON	Recovery (%) OFF	Ratio (ON/OFF)
CaCV					
1.88×10^7	$1.18 \times 10^5 \pm 8.4 \times 10^4$	73	0.63%	0.00039%	1620
1.99×10^6	$7.36 \times 10^3 \pm 2.16 \times 10^3$	~5	0.37%	~0.00024%	~1520
9.93×10^5	$1.66 \times 10^3 \pm 4.63 \times 10^2$	not detected	0.17%	NA	NA
2.20×10^5	8.11×10^{2a}	not detected	0.37%	NA	NA
1.56×10^4	$1.65 \times 10^3 \pm 9.67 \times 10^2$	not detected	10.60%	NA	NA
1.87×10^3	$3.86 \times 10^2 \pm 1.27 \times 10^2$	not detected	21%	NA	NA
RRV					
2.23×10^6	$7.54 \times 10^3 \pm 6.74 \times 10^2$	~2-3	0.34%	~0.00011%	~3000
4.85×10^5	$6.40 \times 10^2 \pm 86$	Not detected	0.13%	NA	NA
9.13×10^4	41 ± 21	Not detected	0.05%	NA	NA
Influenza virus					
4.30×10^6	$3.33 \times 10^3 \pm 7.22 \times 10^2$	Not detected	0.08%	NA	NA

Table 1. Collection efficiency of aerosolized CaCV, rhesus rotavirus (RRV) and Influenza A virus in various concentrations as determined by RT-q PCR. a) This experiment was performed only once.

Ionizing air reduces calicivirus and rotavirus infectivity

Next we determined if collected viruses retained their infectivity after being exposed to negative ions and/or after being exposed to the positively charged collector plate. Five mL of cell culture medium (Eagles Minimal Essential Media (Eagles MEM)) containing 1×10^6 peroxidase forming units of rotavirus respectively of CaCV were aerosolized and collected during 40 min to an active collector plate, containing 1 mL of Eagles MEM. CaCV in cell culture medium was also directly exposed to an active and inactive collector plate, without being aerosolized. Viral infectivity was determined essentially as described[30] and the ratio between viral genome copy numbers versus infectivity was compared between aerosolized virus, virus exposed to active- and inactive collector plates and the viral stocks. CaCV exposed to an active collector plate, without being aerosolized, showed a slight reduction in infectivity (~40%) in comparison to virus that have been trapped on an inactive collector plate (Table 2). In contrast, the infectivity of aerosolized viruses was greatly reduced by >97%, indicating that ionization of the aerosol accounts for the vast majority of infectivity reduction, and not the exposure to the charged collector plate.

Table 2

Reduction of infectivity of Canine Calicivirus (CaCV) and Rhesus Rotavirus (RRV).

	Ratio of infectious virus particles to virus genes per PCR-reaction as quantified by RT-qPCR					
	Exposed to charged collector	**Exposed to uncharged collector**	**Reduction of infectivity**	**Aerolized virus**	**Aerolized virus captured**	**Reduction of infectivity**
CaCV	0.74×10^{-4}	1.24×10^{-4}	40.1%	2.96×10^{-2}	$<7.83 \times 10^{-4}$[a]	>97.4%[a]
RRV	n.d.	n.d.	n.d.	4.86×10^{-1}	$<7.66 \times 10^{-3}$[a]	>98.4%[a]

[a]Under detection limit (10 peroxidase forming units/mL) on the infectivity assay.

Further support that ionizing was the mechanism by which viruses lost infectivity comes from experiments were rotavirus was nebulized without ionizing and allowed to be trapped to an inactive collector plate. Collectors were located at 30 cm from the nebulizer. The result concluded that the genome copy versus infectivity ratio was unchanged from that of the viral stock, thus suggesting that inactivation of virus is associated with ionized air.

Ionizing air and electrostatic attraction prevents airborne-transmitted influenza A/Panama virus infection between guinea pigs

Next we took advantage of an established influenza guinea pig model[31,32,33] to study if ionizing air and electrostatic attraction could prevent airborne aerosol and droplet transmitted influenza A/Panama (Pan/99) virus infection between guinea pigs. The airborne/droplet transmission model was established essentially as described[31] using two separate cages with the ionizer placed between the cages (Fig. 3). Four guinea pigs were infected by intranasal route as described with 5×10^3 pfu of Pan/99[31] and placed in cage "A" (Fig. 3). At 30 hours post infection (h p.i.) 4 uninfected guinea pigs were placed in cage "B" 15 cm from the cage with infected animals as illustrated in Fig. 3, with no physical contact. The ionizer was placed between cages "A" and "B". Two identical experiments were performed, one with active ionizer placed between the cages and one with an inactive ionizer.

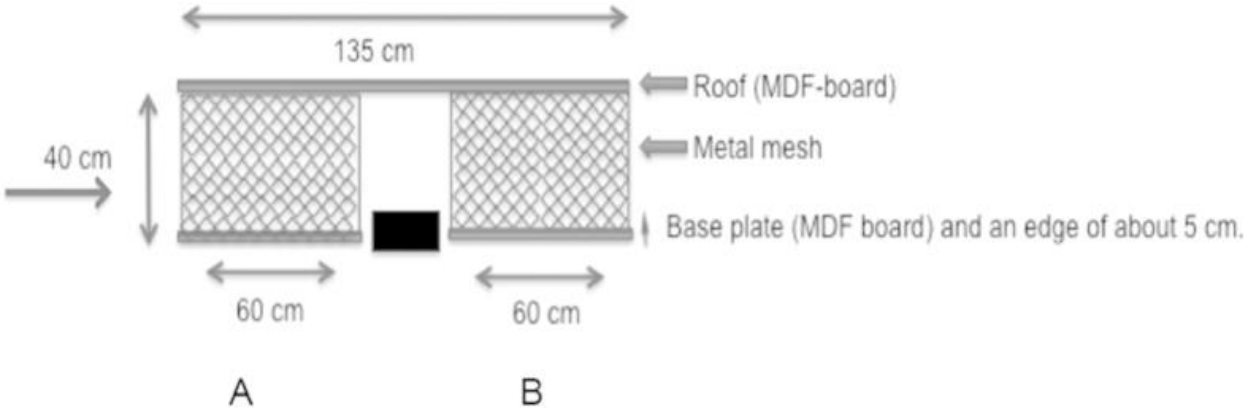

Figure 3

Set-up design of influenza virus (H3N2, Pan/99) aerosol-transmission experiments between guinea pigs. Guinea pigs (n = 4) were intranasally infected with 5×10^3 pfu of Pan/99 virus in 100 uL (50 uL in each nostril).

All four infected animals were placed into an experimental cage "A". At 30 h p.i. four naïve uninfected guinea pigs were placed in cage "B" . Air-flow from left to right. Air exchanged 17x/day. Filled rectangle = ionizer.

Uninfected animals in cage "B" were exposed for 24 hours with airflow from cage "A" hosting the 4 infected guinea pigs and then placed in individually ventilated cages for the next 21 days, to ensure that the only time-point for being infected was the 24 hours when they were exposed to air from infected animals in cage "A". RT-qPCR of lung- and trachea biopsies examined at 54 h p.i. from the nasally experimentally infected animals, revealed that 3 out of 4 guinea pigs in both experiments were positive for influenza.

We assessed transmission of infection from animals in cage "A" to exposed uninfected animals in cage "B" by development of an immune response 21 days post exposure. The results shown in Fig. 4 illustrate that when the ionizer was inactive, 3 of the 4 uninfected but exposed animals developed a serum IgG influenza-specific immune responses. In contrast, none of the 4 animals in cage "B" developed an immune response to influenza virus when the ionizer was active (Fig. 4). Furthermore, influenza virus RNA could be detected by RT-qPCR, albeit at low concentration, on the collector plate from the active ionizer but not with the inactive ionizer, showing that the ionizing device indeed collected virus excreted from the infected animals in cage "A".

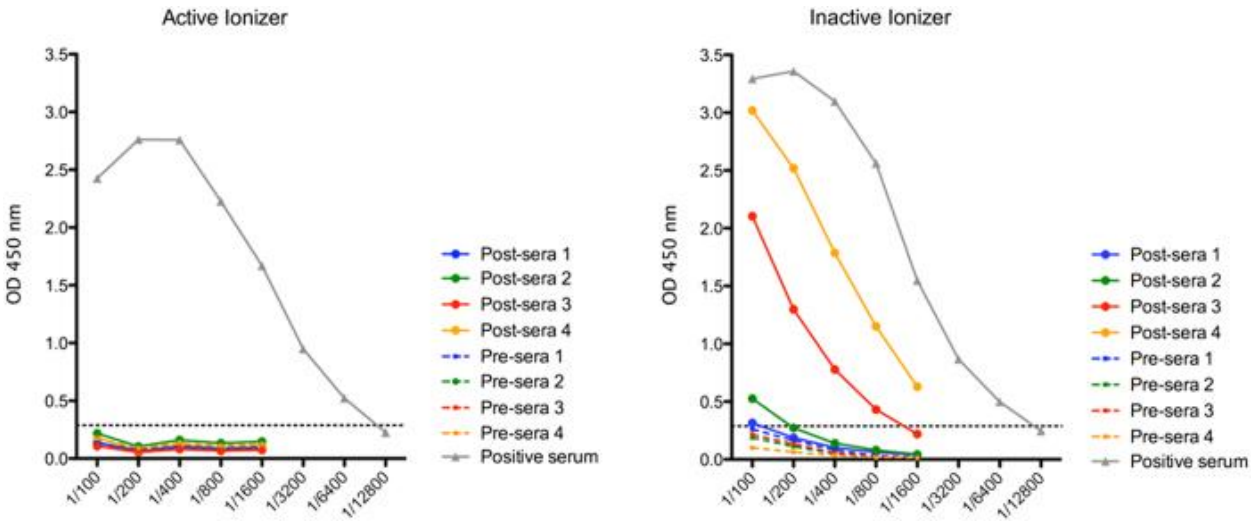

Figure 4

Active ionizer prevents aerosol transmitted influenza virus (H3N2, Pan/99) infection between guinea pigs.

While the active ionizer prevented 4 of 4 exposed guinea pigs from developing an immune response to influenza virus, 3 of 4 animals were infected when the inactive ionizer was used.

Graph shows antibody titers by ELISA before infection (pre-serum 1, 2, 3 and 4) and at day 21 post-exposure to influenza virus (post-serum 1, 2, 3 and 4). Briefly, influenza virus H1N1; (SBL Influenza Vaccine, Sanofi Pasteur, Lyon, France) were coated on ELISA plates and incubated with two-fold dilutions of pre- and post- guinea pig sera, followed by biotinylated rabbit-anti-guinea pig antibody, HRP conjugated streptavidin and TMB substrate as described in Methods. Cut off (dashed line) value (0.284 OD) was the mean of the negative controls +2SD.

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Discussion

We describe a simple ionizing device operating at 12 volt that can prevent spread of airborne transmitted viral infections between animals in a controlled setting, whilst simultaneously collecting virus from air for rapid identification. Coupled with sensitive RT-qPCR assays, this sampling method enabled fast detection and highly sensitive quantification of several human clinically important viruses such as influenza virus, rotavirus and calicivirus. The device consists of a small portable ionizer, where a sampling cup of positive charge is attached to the ionizer attracting negative particles from the air. Important advantages with this novel ionizing device is the simple handling, high robustness as well as the wide applicability to airborne pathogens.

The observation that significantly higher numbers of rotavirus and CaCV particles were detected on the active ionizer compared to the inactive ionizer (~1500–3000 times), led to the conclusion that this technique can actively and efficiently collect viral particles from air. Similarly, visualization of latex particles by SEM revealed that latex particles of all sizes investigated were concentrated on the active collector. It is interesting to note that a broad range of particles sizes, from 35 nm to 10 μm was concentrated, suggesting a wide application range of the technology. However, too large particles may decrease the recovery since these are proposed to remain for less time in the air[33,34].

Interestingly, when we aerosolized low amounts of CaCV, (1.56×10^4 gene copies and 1.87×10^3 gene copies), we observed collection recoveries of 10.6 and 21%, respectively. This markedly increased efficiency, with smaller amounts of virus distribution in air, could be due to less aggregation of virus-virus or virus-cell debris particles more long lasting airborne, and thus leads to stronger electrostatic attraction by the collector. Furthermore, it is likely that much particles end up at the walls of the collector plate or on areas adjacent to the collector plate on the ionizer; and are subsequently not quantified by real-time PCR; thus underestimating the electrostatic effect. When aerolizing higher virus concentrations, this effect can thus lead to lower estimates of recovery. Using CaCV, rotavirus and influenza virus, we performed three independent experiments for each concentration of aerosolized virus in order to assess the robustness of the assay throughout all steps (collection with active ionizer, RNA extraction, cDNA synthesis and real-time PCR). Although several steps are involved from collection to detection we found the assay to be highly robust since the minimum and maximum quantity of virus from each independent measurement was always within a range of 1 log (Fig. 2).

Inactivation of viruses by electrostatic attraction has only been briefly investigated[35]. In the present study, rotavirus and CaCV lost significant (>97%) infectivity (ratio; CaCV from

3.0×10^{-2} to $<7.8 \times 10^{-4}$ and rotavirus from 4.9×10^{-1} to $<7.6 \times 10^{-3}$) in ionized air as determined by a ratio of infectivity versus gene copies. The mechanism of inactivation was not explicitly investigated in this study, but inactivation mechanisms may include reactive species and/or increased protein charge levels, which could inactivate virus as previously described[36,37]. Reduced infectivity has been proposed to be due to reactive oxygen species and ozone, through lipid- and protein peroxidation reactions that may cause damage and destruction to the viral lipid envelope and protein capsid[36]. In particular, protein peroxidation may play a key role in the inactivation of non-enveloped viruses, such as adenovirus, poliovirus and other enteroviruses such as rota- and caliciviruses. Enveloped viruses are suggested to lose infectivity due to lipid peroxidation. However, the cytotoxity of ozone creates a major obstacle for the clinical application of ozone. It has been shown hat increasing the ion concentration of the air efficiently protect chickens from air-born transmission of lethal Newcastle disease virus infection[23]. The exact mechanism of negative ion inactivation of viruses has not been shown and needs to be further investigated. However, in a study using generation of negative and positive ions, influenza virus was inactivated although ozone level was negligible (0.005 ppm or less)[37].

Our device released a steady-state ozone concentration below the detection limit (0.002 ppm) as tested by VTT (Technical Research Center of Finland, Tampere, Finland) and by Air Resources Board in the US, thus ozone cannot in this case be a contributor of viral inactivation. However, reactive radicals such as $\bullet O_2^-$ may be generated, which may contribute to inactivation through damage to either the protein or the nucleic acid structure of the viruses[37]. As infectivity was not lost when virus was nebulized into the air of the room without ionization and only slightly reduced when applied directly on the positively charged collector plate, it is suggested that most reduction of infectivity may be due to increased negative charged levels, presumably resulting in changes in isoelectric point and thus structural changes of the capsid. As the two viruses investigated are non-enveloped, lipid modification can be ruled out.

Most interesting, and of great clinical significance of this study was the novel finding that the ionizing device could detect and prevent influenza virus infection in a controlled setting, mimicking “authentic” conditions. Our intranasal infection protocol was essentially as previously described[31,33] using Harley guinea pigs and 5×10^3 plaque forming units (pfu) of Pan/99 influenza virus. As guinea pigs of the Hartley strain are highly susceptible to human influenza A virus strain Pan/99 (H3N2), with an infectious dose (ID_{50}) of 5 pfu[31], this makes the viral strain most appropriate for these studies. Moreover, Lowen and co-workers have shown 100% transmission of Pan/99 by aerosol to guinea pigs[38,39]. Previous studies have also shown that the used infectious dose results in a viral growth peak around day 3 p.i. in both lungs and nasal passages in this animal model[31], a time point when naïve animals in our study was exposed to air from the infected animals.

We found, by assessing development of the immune response, that 3 of 4 uninfected guinea pigs became infected after exposure to animals inoculated with 5×10^3 pfu of Pan/99. These susceptibility figures are similar to those of Mubareka and co-workers[33] who found that 2 of 3 guinea pigs became infected following short-range aerosol transmission with a dose of 10^3 pfu whereas 3 of 3 animals became infected with a infectious dose of 10^6 pfu. We examined the immune response at 21 days p.i., a time point when Lowen and co-workers[40] previously have found that naturally Pan/99 infected guinea pigs had developed a significant immune response.

The mode of influenza virus transmission includes direct contact with individuals, exposure to virus-contaminated objects (fomites) and inhalation of infectious aerosols. Previous studies using the guinea pig animal model have indicated that aerosol and not fomites is the principal route of Pan/99 transmission between guinea pigs[33]. Aerosol released virus from inoculated animals could be detected on the active collector plate by RT-qPCR, albeit at very low gene copy numbers. Using the guinea pig as a host model, Lowen *et al.*[38] showed that aerosol spread of influenza virus between animals is dependent upon both the relative humidity and temperature. They found that low relative humidity of 20–30% and temperature of 5 °C was most favourable, with no transmission detected at 30 °C. In our set-up system, the temperature was between 20–21 °C and relative humidity between 35–36.2%.

The described ionizing device coupled with RT-qPCR assays has a clear diagnostic potential. The easy handling, low cost, free of ozone production, robustness, high efficiency and low-voltage (12 volt) operation enables large-scale use. Locations critical for infectious spread, such as airplanes, hospitals, day-care centres, school environments and other public places could thus be monitored and controlled by the collection and analysis of airborne viruses and other pathogens on the collector plate. The device also show potential for transmission prevention, although the potency needs to be further investigated in real-life settings. We conclude that this innovative technology hold great potential to collect and identify viruses in environmental air.

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Methods

Study design

The experimental room has grounded metal walls, with a volume of 19 m^3 (B250*L330*H235cm). One active and one inactive ionizer device, designed for collection and analysis of particles in the air, were placed in the room at equal distance (215 cm) from the nebulizer (Aiolos Albatross, Aiolos, Sweden), with a distance of 64 cm between the ionizers. A particle counter (PortaCount Plus, TSI Incorporated, USA) was used before and during the experiment. Before the start of aerosol experiments, the room was emptied on particles by the active ionizer and the collector plate was discarded before the experiments begun and replaced with a new collector plate. The experiments were continued until the particle counts were back to basal level, usually reached within 40 min. Humidity and temperature conditions were measured initial to each aerosol experiments.

Ionizer technology and device

The ionizing device used in this study was developed on the basis of the ion-flow ionizing technology from LightAir AB, Solna, Sweden (www.lightair.com) and was modified for this work by the Department of Microbiology, Karolinska Institute, Stockholm, Sweden. The device (size of 13 × 35 cm) was modified by installing a plastic-cup with a conductive surface of 47 mm in diameter (GP plastindustri, Gislaved, Sweden) as the collector plate (Fig. 1). The collector plate has for safety reasons a very low current, less than 80 μA, however the ionizer accelerates an extremely high voltage of more than 200,000 eV. The ionizer creates electrons, which will

render surface molecules of particles in air negatively charged thus attracting them to the positively charged collector plate. This device generates approximately 35 000 billion electrons per second (www.lightair.com) with a steady-state ozone concentration below the detection limit (0,002 ppm) as tested by VTT Technical Research Center of Finland, Tampere, Finland. It has also been ozone tested and certified by ARB (Air Resources Board) in the US. After the end of the sampling period, the ionizer was turned off, and the collector plate was covered with a lid and stored at −20 °C until analysis. Viruses captured on the collector plates were analyzed by a RT-qPCR for rotavirus, CaCV and influenza virus, and the results from the active- and inactive ionizers were compared. Scanning- and transmission electron microscopy were used for visualization of collected viruses and latex-particles.

Aerosol experiments of virus and latex particles

Different amounts of rhesus rotavirus (genotype G3P[3]), influenza virus (strain H1N1, Salomon Island, inactivated) and CaCV strain 48 (genus *Vesivirus*) were diluted in water to a final volume of 5 mL. In aerosol experiments for scanning electron microscopy and infectivity analysis, virus was diluted in Eagles MEM. Virus suspensions in different concentrations were distributed as aerosols in the room by the use of a nebulizer. Each experiment was performed in triplicates and collection of aerosolized virus and latex particles were performed during 40 min.

Transmission electron microscopy (TEM)

Carbon/Formvar-coated 400 mesh copper grids were placed on the collector during aerosol experiment with influenza- and rotavirus. Grids were then rehydrated in Eagles MEM containing 1% bovine serum albumin (BSA) before being negatively stained with 2% phosphotungstic acid and analyzed by TEM. Ten grid squares were analyzed per specimen and the number of virus particles per unit area was calculated.

Scanning electron microscopy (SEM)

Collected samples were added on the surface of a polycarbonate 0.6 μm filter (Nucleopore, Inc) which was fitted to an airtight gadget (GP Plastindustri AB, Gislaved, Sweden). The filter was dried in room temperature, coated with 40 Å thick layer of ionized gold and analyzed by SEM (Philips High Resolution SEM 515). The method has previously been used and reported in studies of cytomegalovirus as well as cerebrospinal fluid[41,42,43].

Extraction of viral RNA from collector plates

The attached viral particles on the collector plates were lyzed with 1 mL of viral lysis buffer (buffer AVL, QIAamp viral RNA mini kit) added directly into the collector plate and immediately proceeded for extraction of viral RNA using QIAamp Viral RNA Mini Kit (Cat.no: 52906 Qiagen, Hilden, Germany) according to the manufacturer's instructions. Each sample was eluted with 60 μL of RNase-free water containing 0.04% sodium azide (AVE buffer; Qiagen, Hilden, Germany).

Reversed transcription of extracted viral RNA

28 μL of the extracted viral RNA was mixed with 50 pmol of $Pd(N)_6$ random hexamer primer (GE-Healthcare, Uppsala, Sweden) and quickly chilled on ice for 2 min, followed by the addition of one Illustra Ready-To-Go reverse transcriptase PCR (RT-PCR) bead (GE-Healthcare, Uppsala, Sweden) and RNase-free water to a final volume of 50 μL. For rotavirus, an initially denaturation step at 97 °C for 5 min was performed to denature the dsRNA. The reverse transcription (RT) reaction was carried out for 40 min at 42 °C to produce the cDNA later used for real-time PCR.

Quantitative real-time PCR for rotavirus

Rhesus rotavirus was detected and quantified using a LUX real-time PCR assay as described previously[7]. This real-time PCR uses labeled primers with different fluorophores for each VP6 subgroup and external plasmid standards for semi-quantification[44].

Quantitative real-time PCR for CaCV

CaCV was detected and quantified using a SYBR green assay on a ABI prism 7500 (Applied Biosystems, Foster City, CA) with primers; (final concentration 200 nM) CaCV-3 (5-ACCAACGGAGGATTGCCATC-3´ (nucleotides 393 to 410 according to GenBank accession no. AF053720) and CaCV-4 (5´-TAGCCGATCCCACAAGAAGACA-3´ (nucleotides 452 to 474), specific for CaCV strain 48. The reaction was performed with 2 μL cDNA in 10 μL 2X SYBR Green PCR Master Mix (Applied Biosystems) and water to a final volume of 20 μL. The following cycling program was used: 95 °C for 10 min followed by 45 cycles of 95 °C for 15 seconds and 60 °C for 1 min. Melting curve analysis was performed immediately after PCR completion, by heating at 95 °C for 15 seconds, followed by cooling to 60 °C for 1 min and subsequent heating to 95 °C at 0.8 °C min^{-1} with continuous fluorescence recording. Melting temperatures were determined on all samples using the Sequence Detection Software version 1.3.1 (Applied Biosystems) and visualized by plotting the negative derivatives against temperature.

Sampling for infectivity studies with rotavirus and CaCV

To determine whether the ionizing technology has any influence on virus infectivity, rhesus rotavirus and CaCV was aerosolized and collected on active ionizer collector plates, covered with 1 mL of Eagles MEM. Rotavirus (1×10^6 peroxidase forming units) and CaCV (1×10^6 peroxidase forming units) was aerosolized, each in a total volume of 5 mL and collected for 40 min followed by determination of viral infectivity and number of genome copies.

To determine if ionized air *per se* had an effect on viral infectivity, rhesus rotavirus was aerosolized and captured on a collector plate containing 1 mL of Eagles MEM, without ionization, placed at a distance of 30 cm from the nebulizer.

To determine if electrostatic attraction of the collector plate affected viral infectivity, rotavirus (2×10^5 peroxidase forming units) and CaCV (2×10^5 peroxidase forming units) in 1 ml of Eagles MEM, were added to inactive and active collector plates for 40 min. The plates were

subsequently stored at −20 °C until determination of viral infectivity and number of genome copies.

Determination of rotavirus and CaCV infectivity

Rotavirus stock and samples were diluted 1:10 in Eagles MEM and subsequently diluted in two-fold dilutions. Determination of viral infectivity was performed as previously described on confluent Green monkey kidney cells (MA104) in 96-well plates[30]. CaCV infectivity was determined essentially as for rotavirus with the modification that samples were added to confluent Madin-Darby Canine Kidney (MDCK) cells in 48-well plates and infectivity determined as previously described[45] and confirmed by RT-qPCR. To determine the reduction of infectivity, the ratio of viral genome copy numbers versus infectivity was compared between aerosolized virus, virus exposed to active- and inactive collector plates and the viral stock.

Animals

Guinea pigs, strain Hartley, female, 300–350 g, were housed at Astrid Fagraeus Laboratory, Karolinska Institute, according to approved guidelines from the Board of Agriculture and the Council of Europes Convention on vertebrate animals used for scientific purpose. The experimental protocol was approved by the Animal Ethics Committee in Stockholm (Permit Number: N177/11).

Airborne transmission of influenza virus

We use a guinea pig animal model to investigate whether the ionizing technique could prevent transmission of influenza virus infection, since this model have successfully been used as a model of aerosol transmission studies of influenza virus[31,33]. Human influenza A virus, strain Pan/99 (kindly provided by Peter Palese, New York, USA) was used since this strain has been shown to effectively replicates in the upper respiratory airways and effectively transmit by aerosols but not by fomites in guinea pigs. Female guinea pigs, 300–350 g, strain Hartley, were housed at Astrid Fagreaus Laboratorium, Solna, Stockholm (Ethical permission N177/11). Four animals were anesthetized by an intra peritoneal injection of ketamin (Ketalar el Ketaminol) 50 mg/kg and xylazin (Rompun) 5 mg/kg and infected intranasally with 5×10^3 pfu of Pan/99 virus in 100 uL (50 uL in each nostril). All four infected animals were placed into the experimental cage (Fig. 3, cage “A”). At 30 h p.i., four naïve uninfected guinea pigs were placed next to the transmission cage (Fig. 3, cage “B”) at a distance of 15 cm. Air flowed freely between cages, but direct contact between inoculated and exposed animals was prohibited.

The four naïve guinea pigs were exposed for 24 hours and then put into separately individually ventilated cages, to ensure that no aerosol transmission occurred between the animals. Two identical experiments were performed, with an active and inactive ionizer. The nasally infected animals were removed after the exposure time and lung and trachea biopsies were collected (54 h p.i.) and investigated for influenza virus by RT-qPCR. At 21 days post exposure, serum was collected from the uninfected exposed guinea pigs and the prevalence of antibodies against influenza A virus was determined by ELISA. Sera taken before exposure to the infected guinea pigs (pre-sera), and 21 days days after exposure (post-sera) were analyzed from each animal.

ELISA detection of influenza A antibodies

Briefly, 96-well plates (Nunc, 96 F MAXISORP, Roskilde, Denmark) where coated with formalin-inactivated Influenza A virus H1N1 (SBL Influenza Vaccine, Sanoil Pasteur, Lyon, France) diluted in coating buffer (0.05 M sodium carbonate buffer, pH 9.5–9.7) at 5 μg/mL and incubated at +4 °C over night. Wells were washed x3 (0.9% NaCl and 0.05% Tween-20) and blocked with 3% BSA in PBS buffer for 1 hour at 37 °C. Serum samples were diluted 1:100 and further in two-fold dilutions in dilution buffer (PBS containing 0.5% BSA and 0.05% Tween-20), and incubated for 90 min at 37 °C. Plates were then washed x5 and incubated for 60 min at 37 °C with secondary biotinylated goat-anti guinea pig antibody (Vector, BA-7000) and horseradish-peroxidase (HRP) conjugated Streptavidin (DAKO, Denmark, P0397), both at a dilution of 1:3000. Plates were then washed x5 and 100 μL of tetramethyl benzidine (TMB) substrate (Sigma Aldrich, T-0440-16) was added to each well, the reaction developed for 10 min and stopped by addition of 100 μL of 2 M H_2SO_4. Absorbance was measured at 450 nm in an ELISA reader (VersaMax, Molecular Devices). Cut off values were calculated as the average value of negative controls OD and 2 times the SD.

Extraction of influenza RNA from guniea pig tissue

RNA was extracted from trachea and lung tissue of infected guinea pig. Briefly, 100–250 mg of tissue were homogenized with a tissue homogenizer and total RNA extracted with RNAeasy Midi Kit (Qiagen) according to the manufacturer's instructions.

Quantitative real-time PCR for influenza virus

To detect and quantify influenza A virus on the collector plates as well as in guinea pig tissue samples, we used a One-Step Taq Man real-time RT-PCR assay[46] with primers F1-mxA (150 nM) (5′-AAGACCAATYCTGTCACCTCTGA-3′), F3-mxA (150 nM) (5′-CAAGACCAATCTTGTCACCTCT GAC-3′) and R1-mxA (900 nM) (5′-TCCTCGCTCACTGGGCA - 3′) and probes P1-Mx (110 nM) (5′-FAM-TTGTGTTCACGCTCACC–MGB–3′) and P2-Mx (110 nM) (5′-FAM-TTTGTATTCACGCTCACCG–MGB -3′), with the Rotor-Gene Probe RT-PCR Kit (Qiagen). The real-time PCR reaction was performed in a Corbett Rotor-Gene 6000 (Qiagen) with the following cycling protocol: 50 °C for 10 min, followed by 45 cycles of 95 °C for 5 seconds and 57 °C for 15 seconds.

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Additional Information

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Footnotes

Author Contributions L.S., H.W., R.N., M.H. and J.N. designed the experiments, R.N. developed the ionizer device, prepared the set-up of the experimental room and performed the scanning electron microscopy, L.S., M.H., J.N. and R.N. performed the experiments, K.O.H. performed the transmission electron microscopy, M.H. and J.N. performed the laboratory analysis, L.S. and H.W. interpreted the data, L.S., H.W., M.H. and J.N. wrote the manuscript.

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Photocatalytic disinfection using titanium dioxide: spectrum and mechanism of antimicrobial activity

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Abstract

The photocatalytic properties of titanium dioxide are well known and have many applications including the removal of organic contaminants and production of self-cleaning glass. There is an increasing interest in the application of the photocatalytic properties of TiO_2 for disinfection of surfaces, air and water. Reviews of the applications of photocatalysis in disinfection (Gamage and Zhang 2010; Chong et al., Wat Res 44(10):2997–3027, 2010) and of modelling of TiO_2 action have recently been published (Dalrymple et al. , Appl Catal B 98(1–2):27–38, 2010). In this review, we give an overview of the effects of photoactivated TiO_2 on microorganisms. The activity has been shown to be capable of killing a wide range of Gram-negative and Gram-positive bacteria, filamentous and unicellular fungi, algae, protozoa, mammalian viruses and bacteriophage. Resting stages, particularly bacterial endospores, fungal spores and protozoan cysts, are generally more resistant than the vegetative forms, possibly due to the increased cell wall thickness. The killing mechanism involves degradation of the cell wall and cytoplasmic membrane due to the production of reactive oxygen species such as hydroxyl radicals and hydrogen peroxide. This initially leads to leakage of cellular contents then cell lysis and may be followed by complete mineralisation of the organism. Killing is most efficient when there is close contact between the organisms and the TiO_2 catalyst. The killing activity is enhanced by the presence of other antimicrobial agents such as Cu and Ag.

Keywords: Antimicrobial, Disinfection, Mechanism, Photocatalysis, ROS, TiO_2, Titania
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Introduction

The ability of titanium dioxide (titania, TiO_2) to act as a photocatalyst has been known for 90 years (Renz 1921), and its role in the "chalking" of paint (formation of powder on the surface) is well known (Jacobsen 1949). Interest in the application of the photocatalytic properties of TiO_2 was revived when the photoelectrolysis of water was reported by Fujishima and Honda (1972), and this activity was soon exploited both for the ability to catalyse the oxidation of pollutants (Carey et al. 1976; Frank and Bard 1977) and the ability to kill microorganisms (Matusunga 1985; Matsunaga et al. 1985). Photocatalytic surfaces can be superhydrophilic, which means that water spreads on the surface, allowing dirt to be washed off, and commercial uses include self-cleaning windows (e.g. San Gobain Bioclean™, Pilkington Active™ and Sunclean™; Chen and Poon 2009) and self-cleaning glass covers for highway tunnel lamps (Honda et al. 1998). There are currently over 11,000 publications on photocatalysis. Although an early study showed no improved antimicrobial activity of TiO_2 for disinfection of primary wastewater effluent (Carey and Oliver 1980), many subsequent studies have shown the usefulness of photocatalysis on TiO_2 for disinfection of water (Chong et al. 2010). These include killing of bacteria (Rincón and Pulgarin 2004a) and viruses from water supplies (Sjogren and Sierka 1994), tertiary treatment of wastewater (Araña et al. 2002), purifying drinking water (Wei et al. 1994; Makowski and Wardas 2001), treatment of wash waters from vegetable preparation (Selma et al. 2008) and in bioreactor design to prevent biofilm formation (Shiraishi et al. 1999). TiO_2-coated filters have been used for the disinfection of air (Jacoby et al. 1998; Goswami et al. 1997, 1999; Lin and Li 2003a, b; Chan et al. 2005). The advantage of using photocatalysis along with conventional air filtration is that the filters are also self-cleaning. TiO_2 has also been used on a variety of other materials and applications (Table 1). The potential for killing cancer cells has also been evaluated (reviewed by Blake et al. 1999; Fujishima et al. 2000).

Table 1

Some antimicrobial applications of TiO_2

Uses and applications	Publication
Building materials, e.g. concrete	Guo et al. (2009) Chen and Poon (2009)
Catheters to prevent urinary tract infections	Ohko et al. (2001) Yao et al. (2008c)
Coatings for bioactive surfaces	Ueda et al. (2010)
Dental implants	Suketa et al. (2005) Mo et al. (2007)
Fabrics	Gupta et al. (2008), Kangwansupamonkon et al. (2009), Wu et al. (2009a, b), Yuranova et al. (2006)
Food packaging films	Chawengkijwanich and Hayata (2008)
Lancets	Nakamura et al. (2007)
Metal pins used for skeletal traction	Tsuang et al. (2008)
Orthodontic wires	Chun et al. (2007)

Uses and applications	Publication
Paint	Allen et al. (2008)
Photocatalytic tiles for operating theatres	Fujishima et al. (1997)
	Paschoalino and Jardim (2008)
Plastics	Cerrada et al. (2008)
	Fujishima et al. (1997)
Protection of marble from microbial corrosion	Poulios et al. (1999)
Surgical face masks	Li et al. (2006)
Tent materials	Nimittrakoolchai and Supothina (2008)
TiO_2-coated wood	Chen et al. (2009)
TiO_2-containing paper	Geng et al. (2008)

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In recent years, there has been an almost exponential increase in the number of publications referring to photocatalytic disinfection (PCD), and the total number of publications now exceeds 800 (Fig. 1). Some of the early work was reviewed by Blake et al. (1999) and sections on photocatalytic disinfection have been included in several reviews (Mills and Le Hunte 1997; Fujishima et al. 2000, 2008; Carp et al. 2004); reviews of the use in disinfection of water (McCullagh et al. 2007; Chong et al. 2010) and modelling of TiO_2 action have been published (Dalrymple et al. 2010). In this review, we explore the effects of photoactivated TiO_2 on microorganisms.

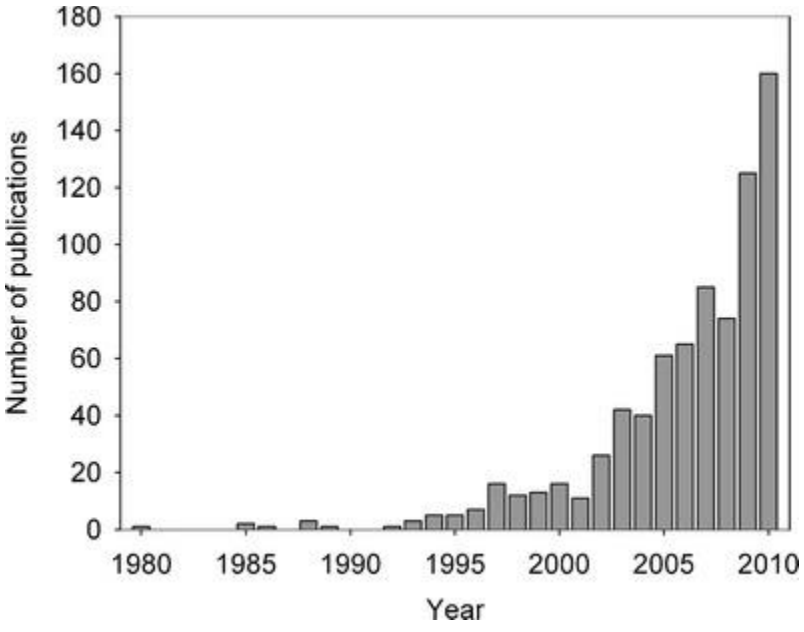

Fig. 1

Number of publications on photocatalytic disinfection

Go to:

Photocatalytic mechanism

For a more detailed discussion of the photochemistry, the reader is directed to the excellent reviews by Mills and Le Hunte (1997) and Hashimoto et al. (2005). TiO_2 is a semiconductor. The adsorption of a photon with sufficient energy by TiO_2 promotes electrons from the valence band (e_{vb}^-) to the conduction band (e_{cb}^-), leaving a positively charged hole in the valence band (h_{vb}^+; Eq. 1). The band gap energy (energy required to promote an electron) of anatase is approx. 3.2 eV, which effectively means that photocatalysis can be activated by photons with a wavelength of below approximately 385 nm (i.e. UVA). The electrons are then free to migrate within the conduction band. The holes may be filled by migration of an electron from an adjacent molecule, leaving that with a hole, and the process may be repeated. The electrons are then free to migrate within the conduction band and the holes may be filled by an electron from an adjacent molecule. This process can be repeated. Thus, holes are also mobile. Electrons and holes may recombine (bulk recombination) a non-productive reaction, or, when they reach the surface, react to give reactive oxygen species (ROS) such as $O_2^{-}\cdot$ (2) and ·OH (3). These in solution can react to give H_2O_2 (4), further hydroxyl (5) and hydroperoxyl (6) radicals. Reaction of the radicals with organic compounds results in mineralisation (7). Bulk recombination reduces the efficiency of the process, and indeed some workers have applied an electric field to enhance charge separation, properly termed photoelectrocatalysis (Harper et al. 2000).

$$TiO_2 + h\nu \rightarrow e_{cb}^- + h_{vb}^+$$
1

$$O_2 + e_{cb}^- \rightarrow O_2^-\cdot$$
2

$$h_{vb}^+ + H_2O \rightarrow \cdot OH + H^+_{aq}$$
3

$$\cdot OH + \cdot OH \rightarrow H_2O_2$$
4

$$O_2^-\cdot + H_2O_2 \rightarrow \cdot OH + OH^- + O_2$$
5

$$O_2^-\cdot + H^+ \rightarrow \cdot OOH$$
6

$$\cdot OH + Organic + O_2 \rightarrow CO_2, H_2O$$
7

There are three main polymorphs of TiO_2: anatase, rutile and brookite. The majority of studies show that anatase was the most effective photocatalyst and that rutile was less active; the

differences are probably due to differences in the extent of recombination of electron and hole between the two forms (Miyagi et al. 2004). However, studies have shown that mixtures of anatase and rutile were more effective photocatalysts than 100% anatase (Miyagi et al. 2004) and were more efficient for killing coliphage MS2 (Sato and Taya 2006a). One active commercially available preparations of TiO_2 is Degussa P25 (Degussa Ltd., Germany) which contains approx. 80% anatase and 20% rutile. The increased activity is generally ascribed to interactions between the two forms, reducing bulk recombination. Brookite has been relatively little studied, but a recent paper showed that a brookite–anatase mixture was more active than anatase alone (Shah et al. 2008). A silver-doped multiphase catalyst was shown to have increased photocatalytic activity, but its antimicrobial activity was not reported (Yu et al. 2005a). Indoor use of photocatalytic disinfection is limited by the requirement for UVA irradiation. Modified catalysts can reduce the band gap so that visible light activates the photocatalysis. This has been shown for TiO_2 combined with C, N and S, metals such as Sn, Pd, and Cu, and dyes (Fujishima and Zhang 2006), but activity is generally lower than when activated with UVA. This area is currently the subject of much research.

The antimicrobial activity of UVA-activated TiO_2 was first demonstrated by Matsunaga and coworkers (Matusunga 1985; Matsunaga et al. 1985). Since then, there have been reports on the use of photocatalysis for the destruction of bacteria, fungi, algae, protozoa and viruses as well as microbial toxins. TiO_2 can be used in suspension in liquids or immobilised on surfaces (Kikuchi et al. 1997; Sunada et al. 1998; Kühn et al. 2003; Yu et al. 2003a; Brook et al. 2007; Yates et al. 2008a, b; Ditta et al. 2008). The ability to eliminate microorganisms on photocatalytic self-cleaning/self-disinfecting surfaces may provide a useful additional mechanism in the control of transmission of diseases along with conventional disinfection methods. Copper and silver ions are well characterised for their antimicrobial activities and can also enhance the photocatalytic activity. Combinations of Cu^{2+} and Ag^{+} with TiO_2 therefore provide dual function surfaces (see below).

Go to:

Photocatalytic action on microorganisms

Photocatalysis has been shown to be capable of killing a wide range of organisms including Gram-negative and Gram-positive bacteria, including endospores, fungi, algae, protozoa and viruses, and has also been shown to be capable of inactivating prions (Paspaltsis et al. 2006). Photocatalysis has also been shown to destroy microbial toxins. As far as the authors are aware, only *Acanthamoeba* cysts and *Trichoderma asperellum* coniodiospores have been reported to be resistant (see below), but these have not been extensively studied. The ability to kill all other groups of microorganisms suggests that the surfaces have the potential to be self-sterilising, particularly when combined with Cu or Ag. However, for the present, it is correct to refer to photocatalytic surfaces or suspensions as being self-disinfecting rather than self-sterilising. Many studies have used pure cultures, although there are reports of photocatalytic activity against mixed cultures (van Grieken et al. 2010) and of natural communities (Armon et al. 1998; Araña et al. 2002; Cho et al. 2007a).

Gram-negative bacteria

The great majority of studies have been performed with *Escherichia coli*, and there are far too many to give a complete list in this review. Some examples of different strains used and applications are shown in Table 2. Examples of other Gram-negative bacteria that are susceptible to PCD are shown in Table 3. They include cocci, straight and curved rods, and filamentous forms from 19 different genera.

Table 2

Examples of *E. coli* strains shown to be killed by photocatalytic disinfection on TiO_2

Organism	Notes	Reference
Escherichia coli	WO_3 nanoparticle doped TiO_2	Tatsuma et al. (2003)
Escherichia coli	Degussa P25 inpregnated cloth filter	Vohra et al. (2006)
Escherichia coli ATCC 8739	Degussa P25 suspension	Cho et al. (2005)
Escherichia coli ATCC 11229	Degussa P25 coated plexiglass	Kühn et al. (2003)
Escherichia coli ATCC 13706	Degussa P25 immobilised on glass substrate	Rodriguez et al. (2007)
Escherichia coli ATCC 10536	Ag and CuO – TiO_2 hybrid catalysts	Brook et al. (2007), Ditta et al. (2008)
Escherichia coli ATCC 15153	Degussa P25 suspension	Ibáñez et al. (2003)
Escherichia coli ATCC 23505	Rfc sputter was used to deposit films of 120 nm thickness onto glass and steel substrates	Shieh et al. (2006)
Escherichia coli ATCC 23631	Degussa P25 applied to a plastic support	Sichel et al. (2007a)
Escherichia coli ATCC 25922	Aldrich TiO_2 99.9% pure anatase	Sökmen et al. (2001)
Escherichia coli ATCC 25922	Aerosol deposited nanocrystalline film	Ryu et al. (2008)
Escherichia coli ATCC 27325	Degussa P25, suspension	Huang et al. (2000) Maness et al. (1999)
Escherichia coli ATCC-39713	Aerosil P25 suspension	Matsunaga et al. (1995)
Escherichia coli CAH57 (ESBL)	Thin film TiO_2	Dunlop et al. (2010)
Escherichia coli CCRC 10675	TiO_2 and ZnO suspension	Liu and Yang (2003)
Escherichia coli CECT 101	Sol–gel microemulsion with an Ag overlayer	Kubacka et al. (2008b)

Organism	Notes	Reference
Escherichia coli DH 4α	Degussa P25 suspension	Lan et al. (2007)
Escherichia coli DH5α	Flow through reactor	Belhácová et al. (1999)
	Anatase thin film on glass	Yu et al. (2002, 2003b)
Escherichia coli HB101	Degussa P25 suspension	Bekbölet and Araz (1996), Bekbölet (1997)
Escherichia coli HB101	Degussa P25 and Ag/P25 mixed suspension	Coleman et al. (2005)
Escherichia coli IFO 3301	Silica coated lime glass plates dip coated with TiO_2	Kikuchi et al. (1997) Sunada et al. (2003b)
Escherichia coli IM303	TiO_2 coated air filter	Sato et al. (2003)
Escherichia coli JM109	Anatase thin film on glass	Yu et al. (2002)
Escherichia coli K12 ATCC10798	Degussa P25 suspension	Duffy et al. (2004) McLoughlin et al. (2004a, b) Pal et al. (2007)
Escherichia coli K12 ATCC10798	Degussa P25 coated glass fibre air filter	Pal et al. (2008)
Escherichia coli K12 (ATCC 23716)	Degussa P25	Rincon and Pulgarin (2003, 2004a)
Escherichia coli K12 (ATCC 2363)	Degussa P25 suspension	Marugan et al. (2008)
Escherichia coli K12	Degussa P25 suspension	Fernandez et al. (2005) Gumy et al. (2006a, b) Quisenberry et al. (2009)
Escherichia coli K12	Thin film TiO_2	Dunlop et al. (2002)
Escherichia coli MG1655	Degussa P25 suspension	Gogniat and Dukan (2007)
Escherichia coli MM294	Degussa P25 suspension	Kim et al. (2004)
Escherichia coli NCIMB-4481	Immobilised TiO_2	Butterfield et al. (1997)
Escherichia coli PHL1273	Degussa P25 suspension	Benabbou et al. (2007)
Escherichia coli PHL1273	Degussa P25 and millennium PC500	Guillard et al. (2008)
Escherichia coli S1400/95	Degussa P25 suspension	Robertson et al. (2005)

Organism	Notes	Reference
Escherichia coli 078	Thin films on glass substrate	Choi et al. (2004)
Escherichia coli XL1 Blue MRF	Anatase thin film on glass	Yu et al. (2002)

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Table 3

Other Gram-negative bacteria shown to be killed by photocatalytic disinfection

Organism	Notes	Reference
Acinetobacter	TiO_2 suspension	Kashyout et al. (2006)
Acinetobacter baumanii	C doped TiO_2	Cheng et al. (2009)
Aeromonas hydrophila AWWX1	TiO_2 pellets	Kersters et al. (1998)
Anabaena	TiO_2-coated glass beads	Kim and Lee (2005)
Bacteroides fragilis	TiO_2 on orthopaedic implants	Tsuang et al. (2008)
Coliforms	Degussa P25 suspension	Araña et al. (2002)
Coliforms	Anatase suspension	Watts et al. (1995)
Edwardsiella tarda	Sol/gel-coated glass slides	Cheng et al. (2008)
Enterobacter aerogenes	Degussa P25 suspension	Ibáñez et al. (2003)
Enterobacter cloacae SM1	Anatase, spin-coated glass plates	Yao et al. (2007a)
Erwinia carotovora subsp. *carotovora*	Degussa P25 suspension	Muszkat et al. (2005)
Erwinia carotovora subsp. *carotovora* ZL1, subsp. *Carotovora 3*, subsp. *Carotovora 7*	Anatase, spin-coated glass lates	Yao et al. (2007a, b, 2008a, b)
Faecal colifoms	Anatase suspension	Watts et al. (1995)
Flavobacterium sp.	TiO_2 suspension and coated glass beads	Cohen-Yaniv et al. (2008)
Fusobacterium nucleatum	Thin film of anatase on titanium	Suketa et al. (2005), Bai et al. (2007)
Legionella pneumophila ATCC 33153	Degussa P25 suspension	Cheng et al. (2007)
Legionella pneumophila CCRC 16084	TiO_2 air filter + UVC	Li et al. (2003)
Legionella pneumophila GIFU-9888	Ultrasonic activated suspension of TiO_2	Dadjour et al. (2005, 2006)
Microcystis	TiO_2-coated glass beads	Kim and Lee (2005)
Porphyromonas gingivalis	TiO_2 sol/gel-coated orthodontic wires	Chun et al. (2007)
Prevotella intermedia	Ag–hydroxyapatite–TiO_2 catalyst	Mo et al. (2007)

Organism	Notes	Reference
Proteus vulgaris	P25 (10% Pt),0.25 g/L slurry	Matsunaga et al. (1985)
P. aeruginosa	Surfaces	Kühn et al. (2003)
P. aeruginosa environmental isolate	Spray-coated soda lime glass and silica tubing	Amezaga-Madrid et al. (2002, 2003)
P. aeruginosa PA01	Thin film	Gage et al. (2005)
P. aeruginosa	Coated Al fibres	Luo et al. (2008)
P. aeruginosa	Catheters	Yao et al. (2008c)
P. fluorescens R2F	TiO_2 pellets	Kersters et al. (1998)
P. fluorescens B22	Sigma-Aldrich TiO_2 thin films	Skorb et al. (2008)
Pseudomonas sp.	Anodized titanium alloy	Muraleedharan et al. (2003)
Pseudomonas stutzeri NCIMB11358	TiO_2 suspension	Biguzzi and Shama (1994)
Pseudomonas syringae pv tomato	Degussa P25 suspension	Muszkat et al. (2005)
Pseudomonas tolaasi	TiO_2 suspension	Sawada et al. (2005)
Salmonella choleraesuis	Anatase suspension	Kim et al. (2003)
Salmonella enteriditis Typhimurium	Degussa P25 suspension	Ibáñez et al. (2003), Cushnie et al. (2009)
Salmonella enteriditis Typhimurium	TiO_2 film on quartz rods with UVC	Cho et al. (2007a, b)
Serratia marcescens	Degussa P25 suspension	Block et al. (1997) Goswami et al. (1999)
Shigella flexneri	C-doped TiO_2	Cheng et al. (2009)
Vibrio parahaemolyticus	Anatase suspension	Kim et al. (2003)
Vibrio parahaemolyticus VP 144	Anatase TiO_2 dip coated on open porcelain filter cell	Hara-Kudo et al. (2006)
Vibrio vulnificus	TiO_2-impregnated steel fibres for water treatment	Song et al. (2008)

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Gram-positive bacteria

Most studies showed that Gram-positive bacteria were more resistant to photocatalytic disinfection than Gram-negative bacteria (Kim et al. 2003; Liu and Yang 2003; Erkan et al. 2006; Pal et al. 2005, 2007; Muszkat et al. 2005; Hu et al. 2007; Sheel et al. 2008; Skorb et al. 2008). The difference is usually ascribed to the difference in cell wall structure between Gram-positive and Gram-negative bacteria. Gram-negative bacteria have a triple-layer cell wall with an inner membrane (IM), a thin peptidoglycan layer (PG) and an outer membrane (OM), whereas

Gram-positive bacteria have a thicker PG and no OM. However, a few studies show that Gram-positive bacteria were more sensitive. *Lactobacillus* was more sensitive than *E. coli* on a Pt-doped TiO_2 catalyst (Matsunaga et al. 1985). methicillin-resistant *Staphylococcus aureus* (MRSA) and *E. coli* were more resistant than *Micrococcus luteus* (Kangwansupamonkon et al. 2009). Dunlop et al. (2010) showed that MRSA were more sensitive than an extended spectrum β-lactamase (ESBL)-producing *E. coli* strain, but less sensitive than *E. coli* K12. *Enterococcus faecalis* was more resistant than *E. coli*, but more sensitive than *Pseudomonas aeruginosa* (Luo et al. 2008). Conversely, Kubacka et al. (2008a) showed no difference in sensitivity between clinical isolates of *P. aeruginosa* and *E. faecalis.* Van Grieken et al. (2010) saw no difference in disinfection time for *E. coli* and *E. faecalis* in natural waters, but *E. faecalis* was more resistant in distilled water. These differences may relate to different affinities for TiO_2 (close contact between the cells and the TiO_2 is required for optimal activity—see below) as well as cell wall structure.

Gram-positive bacteria that have been shown to be killed by PCD are shown in Table 4 and include species of 17 different genera, including aerobic and anaerobic endospore formers. The endospores were uniformly more resistant than the vegetative cells to PCD.

Table 4

Gram-positive bacteria shown to be killed by photocatalytic disinfection

Organism	Notes	Reference
Actinobacillus actinomycetemcomitans	TiO_2 coating on titanium	Suketa et al. (2005)
Actinomyces viscosus	Kobe Steel TiO_2 99.98% anatase	Nagame et al. (1989)
Bacillus cereus	TiO_2 suspension	Cho et al. (2007a)
Bacillus cereus spores	TiO_2 suspension	Armon et al. (2004)
Bacillus megaterium QM B1551	Colloidal suspension of TiO_2	Fu et al. (2005)
Bacillus pumilis spores ATCC 27142	TiO_2 anatase 99.9% slurry in Petri dish	Pham et al. (1995, 1997)
Bacillus sp.	Degussa P-25 immobilised on Pyrex glass	Rincón and Pulgarin (2005)
Bacillus subtilis vegetative cells and endospores	Degussa P25-coated quartz discs	Wolfrum et al. (2002)
Bacillus subtilis endospores	Aluminium foil coated with TiO_2	Greist et al. (2002)
Bacillus thuringiensis	100% anatase thin film ± Pt doping	Kozlova et al. (2010)
Clavibacter micheganensis	Solar + H_2O_2	Muszkat et al. (2005)
Clostridium difficile	Evonik Aeroxide P25 thin fim	Dunlop et al. (2010)
Clostridium perfringens spores NCIMB 6125	TiO_2 film on metal electrode	Butterfield et al. (1997)

Organism	Notes	Reference
Clostridium perfringens spores	Degussa P-25 + UVC	Guimarães and Barretto (2003)
Deinococcus radiophilus	TiO_2 suspension	Laot et al. (1999)
Enterococcus (Streptococcus) faecalis	Degussa P25 suspension	Herrera Melián et al. (2000)
Enterococcus (Streptococcus) faecalis	Immobilised TiO_2	Singh et al. (2005)
Enterococcus faecalis CECT 481	Degussa P25 suspension	Vidal et al. (1999)
Enterococcus faecium	Degussa P25-coated Plexiglass	Kühn et al. (2003)
Enterococcus hirae	TiO_2 on orthopaedic implants	Tsuang et al. (2008)
Enterococcus sp.	Degussa P-25 suspension	Rincón and Pulgarin (2005)
Lactobacillus acidophilus	Degussa P25 suspension	Matsunaga et al. (1985), Choi et al. (2007a)
Lactobacillus helveticus CCRC 13936	TiO_2 suspension	Liu and Yang (2003)
Lactococcus lactis 411	Sigma-Aldrich TiO_2 thin films	Skorb et al. (2008)
Listeria monocytogenes	TiO_2 (Yakuri Pure Chemical Company, Japan) suspension	Kim et al. (2003)
Microbacterium sp. Microbacteriaceae str. W7	Degussa P25 immobilised on membrane	Pal et al. (2007)
Micrococcus luteus	Degussa P25 thick film	Wolfrum et al. (2002)
Micrococcus lylae	TiO_2 suspension	Yu et al. (2005b)
MRSA	Fe_3O_4–TiO_2 core/shell magnetic nanoparticles in suspension	Chen et al. (2008)
MRSA	TiO_2 thin film on titanium	Oka et al. (2008)
Mycobacterium smegmatis	100% anatase thin film ± Pt doping	Kozlova et al. (2010)
Porphyromonas gingivalis	TiO_2 thin film on steel and titanium	Shiraishi et al. (1999)
Paenibacillus sp SAFN-007	Degussa P25 immobilised on membrane	Pal et al. (2007)
Staphylococcus aureus	Degussa P25 suspension	Block et al. (1997)
Staphylococcus aureus	TiO_2 thin film on steel and titanium	Shiraishi et al. (1999)
Staphylococcus epidermidis NCTC11047	Ag-TiO_2 catalyst	Sheel et al. (2008)
Staphylococcus saprophyticus	Fe_3O_4–TiO_2 core/shell magnetic nanoparticles in suspension	Chen et al. (2008)
Streptococcus cricetus	Kobe Steel TiO_2 99.98% anatase	Nagame et al. (1989)

Organism	Notes	Reference
Streptococcus iniae	Sol/gel-coated glass slides	Cheng et al. (2008)
Streptococcus mutans	TiO_2 sol/gel-coated orthodontic wires	Chun et al. (2007)
Streptococcus mutans GS5, LM7, OMZ175	P25 aerosil, 70% anatase suspension	Saito et al. (1992)
Streptococcus pyogenes $ery^r cam^r$	Fe_3O_4–TiO_2 core/shell magnetic nanoparticles in suspension	Chen et al. (2008)
Streptococcus rattus FA-1	P25 aerosil, 70% anatase suspension	Saito et al. (1992)
Streptococcus sobrinus AHT	P25 suspension	Saito et al. (1992)

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Fungi, algae and protozoa

Fungi, algae and protozoa that have been shown to be susceptible to PCD are shown in Tables 5 and and6.6. These include 11 genera of filamentous fungi, 3 yeasts, 2 amoebae, 1 Apicomplexan, 1 diplomonad, 1 ciliate and 7 algae, including 1 diatom. Fungal spores were generally more resistant than vegetative forms, and *Trichoderma harzianum* spores in particular were resistant to killing under the conditions tested (Giannantonio et al. 2009). Cysts of *Acanthamoeba* showed only a 50% reduction during the treatment time and may have been killed if the treatment time had been extended (Sökmen et al. 2008).

Table 5

Fungi shown to be killed by photocatalytic disinfection

Organism	Notes	Reference
Aspergillus niger AS3315	Wood coated with TiO_2	Chen et al. (2009)
A. niger spores	Degussa P25 film on quartz discs	Wolfrum et al. (2002)
Aspergillus niger	Thin films of TiO_2 on glass plates	Erkan et al. (2006)
Candida albicans ATCC 10231	Degussa P25 suspension	Lonnen et al. (2005)
Candida albicans	TiO_2-coated surfaces	Kühn et al. (2003)
Candida famata	TiO_2 coated catheters	Yao et al. (2008c)
Candida vini	TiO_2 thin film	Veselá et al. (2008)
Cladobotryum varium	TiO_2 suspension	Sawada et al. (2005)
Cladosporium cladospoiroides	TiO_2-coated concrete	Giannantonio et al. (2009)
Diaporthe actinidae	TiO_2 immobilised on alumina spheres	Hur et al. (2005)

Organism	Notes	Reference
Erysiphe cichoracearum	Degussa P25 and Ce^{3+} doped catalysts	Lu et al. (2006)
Epicoccum nigrum	TiO_2 coated concrete	Giannantonio et al. (2009)
Fungi from spinach	Plastic fruit containers with TiO_2 coating	Koide and Nonami (2007)
Fusarium mucor	TiO_2-coated concrete	Giannantonio et al. (2009)
Fusarium solani ATCC 36031	Degussa P25 suspension	Lonnen et al. (2005)
Fusarium spp. (*equisetii, oxypartan, anthophilum, verticilloides, solani*)	TiO_2 suspension, solar irradiation	Sichel et al. (2007b, c)
Hanseula anomala CCY-138-30	TiO_2- and Ag-doped	Veselá et al. (2008)
Peronophythora litchii	Degussa P25- and Ce^{3+}-doped catalysts	Lu et al. (2006)
Penicillium citrinum	TiO_2-coated air filter	Lin and Li (2003a, b)
Penicillium expansum	TiO_2 spray coated on polypropylene film	Maneerat and Hayata (2006)
Penicillium oxalicum	TiO_2-coated concrete	Giannantonio et al. (2009)
Pestaotiopsis maculans	TiO_2-coated concrete	Giannantonio et al. (2009)
Saccharomyces cerevisiae	Aerosil P25 suspension	Matsunaga et al. (1985)
Sacchararomyces cerevisiae	Pd-doped TiO_2	Erkan et al. (2006)
Spicellum roseum	TiO_2 suspension	Sawada et al. (2005)
Trichoderma asperellum	TiO_2-coated concrete	Giannantonio et al. (2009)
Trichoderma harzianum	TiO_2 suspension	Sawada et al. (2005)

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Table 6

Protozoa and algae shown to be killed by photocatalytic disinfection

Organism	Notes	Reference
Protozoa		
Acanthamoeba castellanii	Degussa P25 suspension Only 50% kill for cysts, trophozoites were sensitive	Sökmen et al. (2008)

Organism	Notes	Reference
Acanthamoeba polyphaga environmental isolate	Degussa P25 suspension	Lonnen et al. (2005)
Cryptosporidium parvum	UVC + TiO_2	Ryu et al. (2008)
Cryptosporidium parvum	Sol–gel and thermal TiO_2 thin films applied to Petri dish with a counter electrode Pt mesh	Curtis et al. (2002)
Giardia sp.	Fibrous ceramic TiO_2 filter	Navalon et al. (2009)
Giardia intestinalis cysts	TiO_2 (anatase 99.9%) + Ag^+	Sökmen et al. (2008)
Giardia lamblia	TiO_2 thin film catalyst	Lee et al. (2004)
Tetrahymena pyriformis	TiO_2 suspension	Peng et al. (2010)
Algae		
Amphidinium corterae	Ag–TiO_2 catalyst	Rodriguez-Gonzalez et al. (2010)
Chlorella vulgaris	TiO_2–Pt catalyst	Matsunaga et al. (1985)
Cladophora sp.	TiO_2-covered glass beads	Peller et al. (2007)
Chroococcus sp. 27269	Anatase, fluorescent light	Hong et al. (2005)
Melosira sp.	TiO_2-coated glass beads	Kim and Lee (2005)
Oedogonium sp.	TiO_2-coated concrete	Linkous et al. (2000)
Tetraselmis suecica	Ag–TiO_2 catalyst	Rodriguez-Gonzalez et al. (2010)

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Viruses

Viruses that have been shown to be killed by PCD are shown in Table 7.

Table 7

Viruses shown to be killed by photocatalytic disinfection

Host	Virus	Reference
Bacteroides fragilis	Not specified	Armon et al. (1998)
Birds	Influenza (avian) A/H5N2	Guillard et al. (2008)

Host	Virus	Reference
E. coli	Coliphage	Guimarães and Barretto (2003)
E. coli	*fr*	Gerrity et al. (2008)
E. coli	T4	Ditta et al. (2008), Sheel et al. (2008)
E. coli	λ vir	Yu et al. (2008)
E. coli	λNM1149	Belhácová et al. (1999)
E. coli	φX174	Gerrity et al. (2008)
E. coli	MS2	Sjogren and Sierka (1994), Greist et al. (2002), Cho et al. (2004, 2005), Sato and Taya (2006a, b), Vohra et al. (2006), Gerrity et al. (2008)
E. coli	Qβ	Lee et al. (1997), Otaki et al. (2000)
Human	Hepatitis B virus surface antigen HBsAg	Zan et al. (2007)
Human	Influenza A/H1N1	Lin et al. (2006)
Human	Influenza A/H3N2	Kozlova et al. (2010)
Human	Norovirus	Kato et al. (2005)
Human	*Poliovirus* type 1 (ATCC VFR-192)	Watts et al. (1995)
Human	SARS coronavirus	Han et al. (2004)
Human	Vaccinia	Kozlova et al. (2010)
Lactobacillus casei	PL-1	Kakita et al. (1997, 20000, Kashige et al. (2001)
Salmonella typhimurium	PRD1	Gerrity et al. (2008)

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Most studies were on *E. coli* bacteriophages in suspension, which have been demonstrated for icosahedral ssRNA viruses (MS2 and Qβ), filamentous ssRNA virus (fr), ssDNA (phi-X174) and dsDNA viruses (λ and T4). Other bacteriophages include *Salmonella typhimurium* phage PRD-1, *Lactobacillus* phage PL1 and an unspecified *Bacteroides fragilis* phage. Mammalian viruses include poliovirus 1, avian and human influenza viruses, and SARS coronavirus (Table 7).

Bacterial toxins

Photocatalytic activity has been shown to be capable of inactivating bacterial toxins including Gram-negative endotoxin and algal and cyanobacterial toxins (Table 8).

Table 8

Microbial toxins inactivated by photocatalysis

Toxin	Publication
Brevetoxins	Khan et al. (2010)
Cylindrospermopsin	Senogles et al. (2000, 2001)
Lipopolysaccharide endotoxin	Sunada et al. (1998)
Microcystin-LR	Lawton et al. (1999, 2003)
	Cornish et al. (2000)
	Feitz and Waite (2003)
	Choi et al. (2007b)
Microcystins LR, YA and YR	Shephard et al. (1998)
Nodularin	Liu et al. (2005)

Go to:

Mechanism of killing of bacteria

The mode of action of photoactivated TiO_2 against bacteria has been studied with both Gram-positive and Gram-negative bacteria. The killing action was originally proposed to be via depletion of coenzyme A by dimerization and subsequent inhibition of respiration (Matsunaga et al. 1985, 1988). However, there is overwhelming evidence that the lethal action is due to membrane and cell wall damage. These studies include microscopy, detection of lipid peroxidation products, leakage of intercellular components, e.g. cations, RNA and protein, permeability to low-molecular-weight labels, e.g. *o*-nitrophenyl-galactoside (ONPG), and spectroscopic studies.

Changes in cell permeability

Indirect evidence for membrane damage comes from studies of leakage of cellular components. Saito et al. (1992) showed that there was a rapid leakage of K^+ from treated cells of *Streptococcus sobrinus* AHT which occurred within 1 min of exposure and paralleled the loss of viability. This was followed by a slower release of RNA and protein. Leakage of K^+ was also shown to parallel cell death of *E. coli* (Hu et al. 2007; Kambala and Naidu 2009). Huang et al. (2000) showed an initial increase in permeability to small molecules such as ONPG which was followed by leakage of large molecules such as β-d-galactosidase from treated cells of *E. coli*, suggesting a progressive increase in membrane permeability. Membrane damage has been shown with cells labelled with the LIVE-DEAD® BacLight™ Bacterial Viability Kit which uses the fluorescent dyes Cyto 9, which stains all cells green, and propidium iodide, which only penetrates cells with damaged membranes and stains cells red. Gogniat et al. (2006) showed that permeability changes occurred in the membrane soon after attachment of *E. coli* to the TiO_2, and we have seen similar changes (Ditta and Foster, unpublished). However, no damage was detected on a visible light active PdO/TiON catalyst until the catalyst had been irradiated (Wu et al. 2010b). SEM clearly showed membrane damage after irradiation on this catalyst (Wu et al. 2008, 2009a, b, 2010b; see Fig. 2).

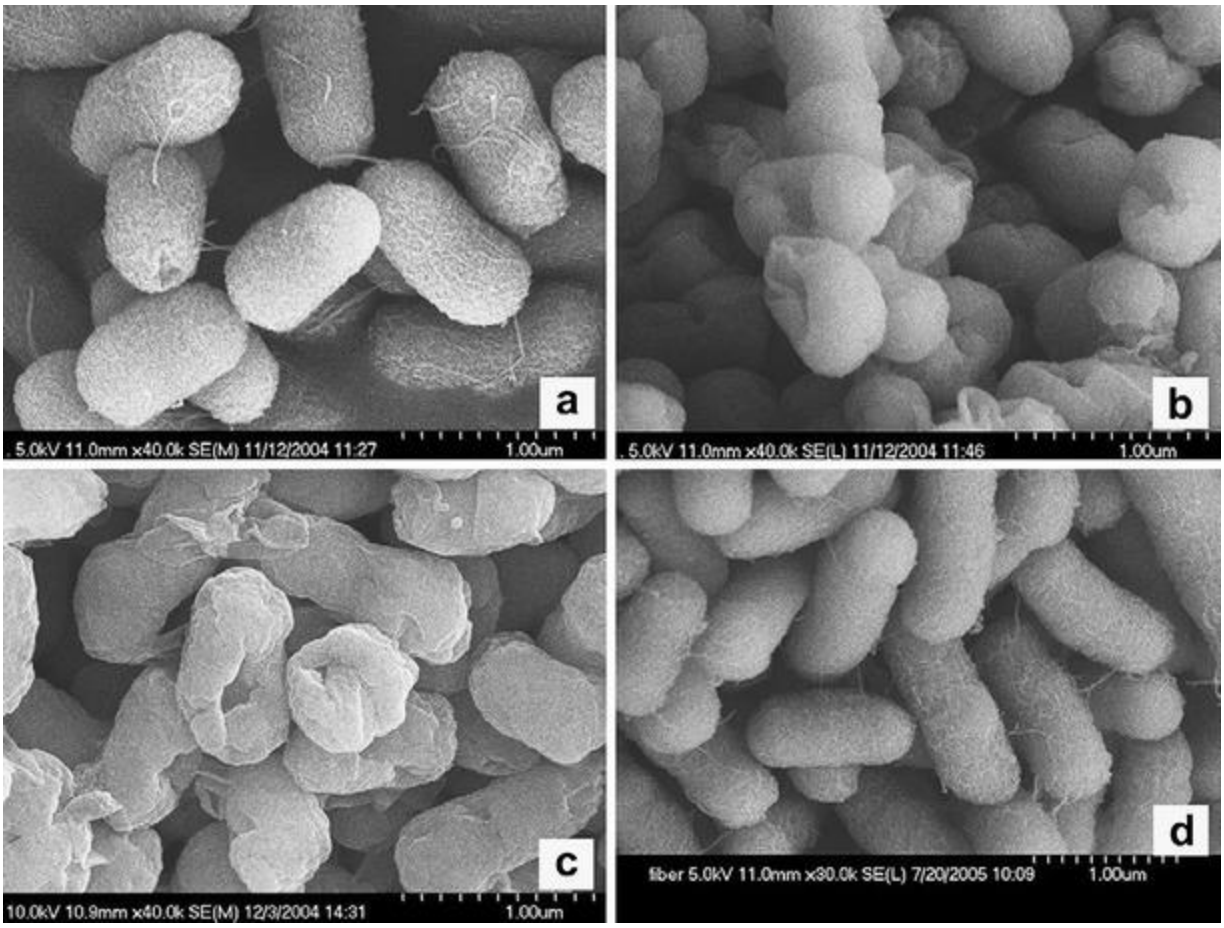

Fig. 2

Scanning electron micrographs of photocatalytically treated *E. coli*. **a** Untreated cells. **b**, **c** Cells after 240 min. **d** Cells after 30 min. Catalyst TiON thin film. From Wu et al. (2010a, b)

Microscopic changes during PCD

TEM images of treated cells of *S. sobrinus* showed clearly that the cell wall was partially broken after cells had undergone TiO_2 photocatalytic treatment for 60 min, with further disruption after 120 min (Saito et al. 1992). The authors suggested that cell death was caused by alterations in cell permeability and the decomposition of the cell wall. SEM images of *S. aureus*, MRSA, *E. coli* and *M. luteus* showed morphological changes suggestive of cell wall disruption after UVA irradiation on apatite-coated TiO_2 on cotton fabrics (Kangwansupamonkon et al. 2009).

Damage to the cell wall of *P. aeruginosa* was shown by SEM and TEM, which showed changes in membrane structure such as “bubble-like protuberances which expelled cellular material” (Fig. 3; Amezaga-Madrid et al. 2002, 2003). They suggested that leakage of cellular material, and possibly abnormal cell division, was occurring, although the bubbles may have been due to localised damage to the peptidoglycan layer allowing the inner membrane to bulge through the peptidoglycan layer. Sunada et al. (2003b) studied killing of *E. coli* on thin films of TiO_2 and

showed that the outer membrane was damaged first and then the cytoplasmic membrane followed by complete degradation. Photocatalytic killing occurred without substantial visible degradation of peptidoglycan. Atomic force microscopy measurements of cells on illuminated TiO_2 film showed that the outer membrane decomposed first (Sunada et al. 2003b).

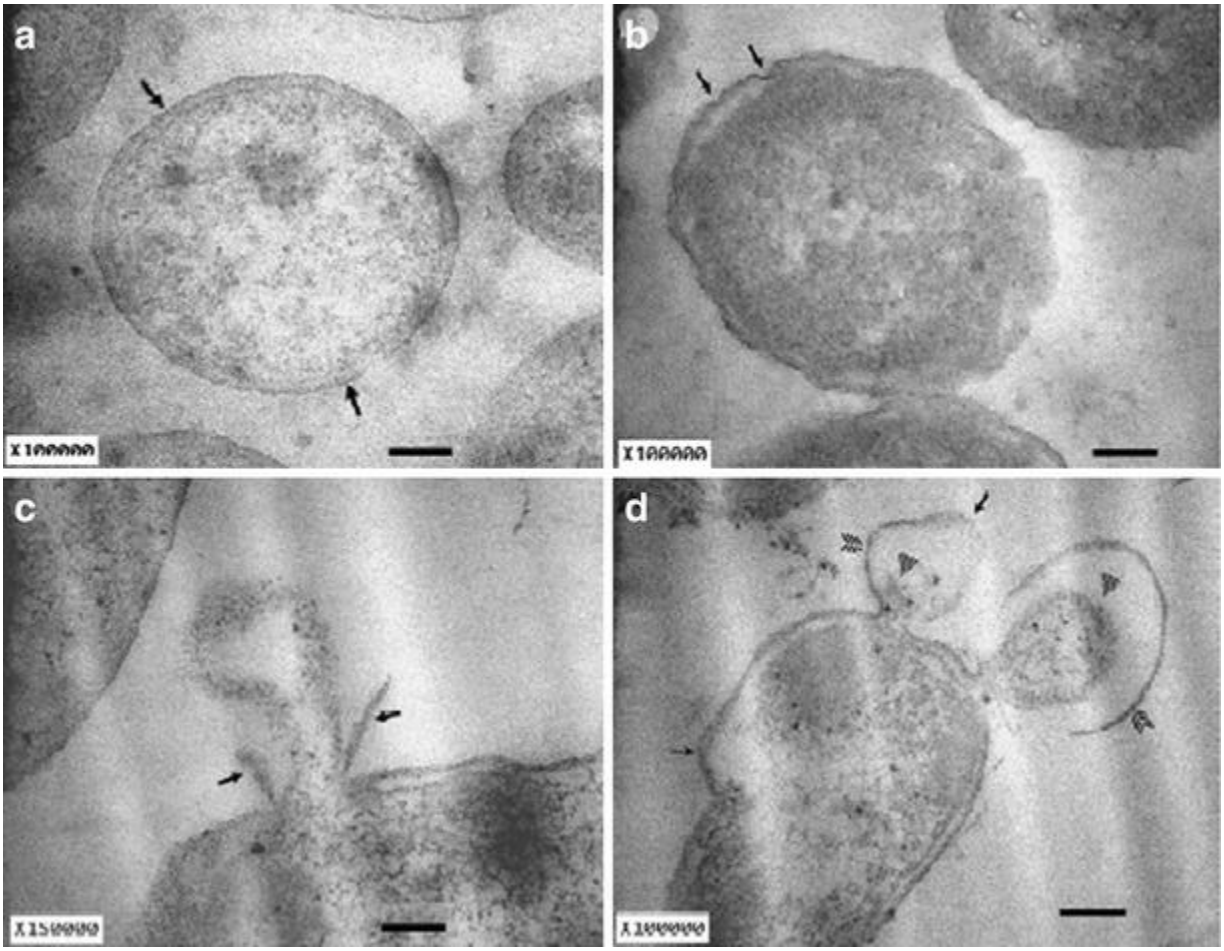

Fig. 3

Transmission electron micrographs of photocatalytically treated *P. aeruginosa*. Untreated cells transverse section showing normal thickness and shape cell wall (*arrows*). **b**–**d** Cells after 240 min treatment showing abnormal wavy cell wall (*arrows*) (**b**), cytoplasmic material escaping from the cell with damaged cell wall (*arrows*) (**c**) and cell showing two "bubbles" of cellular material with cell wall (*arrows*) (**d**). Catalyst TiO_2 thin film. *Bar marker* = 200 nm. From Amezaga-Madrid et al. (2003b)

TEM images showed progressive destruction of *E. coli* cells on $Ag/AgBr/TiO_2$ in suspension (Hu et al. 2006). Cell membrane was degraded first followed by penetration of TiO_2 particles into the cell and further damage. TEM of *E. coli* showed that there were changes to the nucleoid which became condensed, possibly due to leakage of ions out of the cell (Chung et al. 2009).

TEM of thin sections of treated cells of *E. coli* on a visible light-activated TiO_2 showed various degrees of cell disruption including plasmolysis, intracellular vacuoles ghost and cell debris (Vacaroiu et al. 2009). SEM and TEM studies showed initial swelling and rough appearance of the cells followed by scars and holes in the OM, especially where the TiO_2 particles were in contact with the cells. Erdem et al. (2006) showed damage by SEM on *E. coli* and production of membrane breakdown products. SEM has shown changes to the outer membrane of *E. coli* (Li et al. 2008; Shah et al. 2008; Gartner et al. 2009). TEM of thin sections of treated cells of *E. coli* on a visible light-activated TiO_2 showed various degrees of cell disruption including plasmolysis, intracellular vacuoles ghost and cell debris (Vacaroiu et al. 2009).

Atomic force microscopy was used to show membrane damage to *E. coli*, *S. aureus* and *Diplococcus (Streptococcus) pneumoniae* on thin films of TiO_2 (Miron et al. 2005). Changes to treated cells of *S. aureus* seen by TEM included separation of cytoplasmic membrane from the peptidoglycan layer (Chung et al. 2009). Distortion of treated cells of both MRSA and methicillin-sensitive *S. aureus* was seen by SEM on anatase–brookite (Shah et al. 2008), again suggesting cell wall damage.

Lipid peroxidation by ROS was demonstrated by the release of MDA as a breakdown product, and there was a concurrent loss of membrane respiratory activity measured by reduction of 2,3,5-triphenyltetrazolium chloride (Maness et al. 1999). The demonstration of degradation of *E. coli* endotoxin without substantial degradation of peptidoglycan (Sunada et al. 1998) suggested that in the case of Gram-negative bacteria, cell disruption occurred in the order of OM→PG→IM. However, alterations to the peptidoglycan layer may not be obvious in electron micrographs as peptidoglycan is a highly cross-linked structure and appreciable damage may occur without destruction of its overall appearance. Localised destruction may occur where TiO_2 particles are in contact with the cell. This may allow protrusion of inner membrane through the cell wall as seen by Amezaga-Madrid et al. (2003), followed by total rupture of the cell wall.

Yao et al. (2007c) showed damage to cells of *Erwinia carotovora* and DNA damage, which suggested that damage to DNA was responsible for cell death. However, our own data showed that there was no DNA damage seen by COMET assay on plain TiO_2 surfaces even when 97% of the cells were non-viable (Varghese and Foster, unpublished data; Fig. 4). Damage to DNA does occur on TiO_2 (Wamer et al. 1997; Hirakawa et al. 2004; Wang and Yang 2005; Wang et al. 2005; Gogniat and Dukan 2007; Shen et al. 2008; Yao et al. 2007c; Yang and Wang 2008), but is probably a late event after rupture of the membrane and cell death.

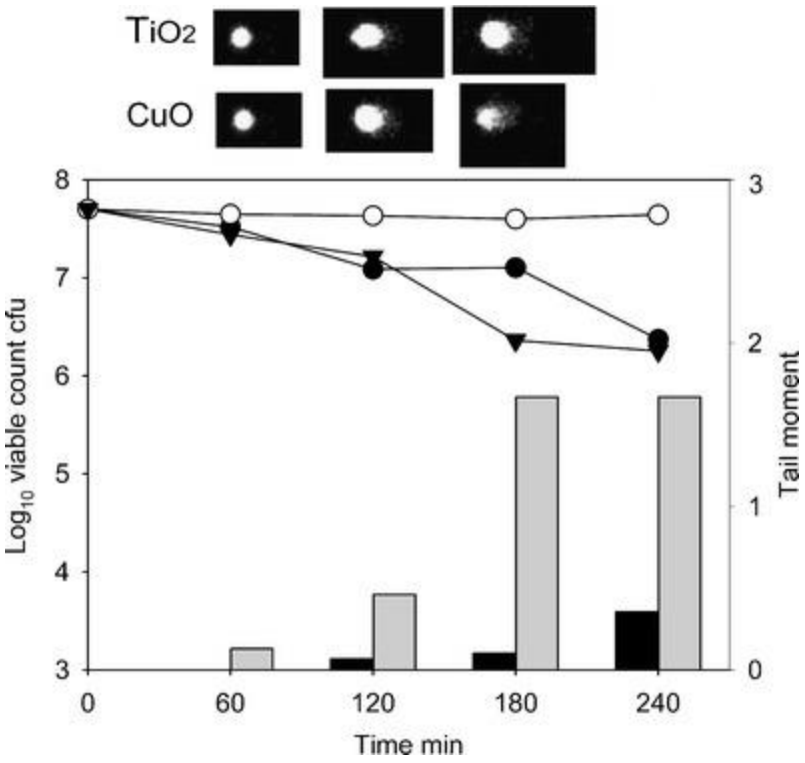

Fig. 4

Comet assay of DNA from cells of *E. coli* on photoirradiated TiO_2 and CuO–TiO_2 catalysts. *Upper photographs* show fragmented DNA entering the gel like the tail of a comet. The graph shows viability (control, *open circle*; TiO_2 catalyst, *closed circle*; TiO_2–CuO dual catalyst, *downturned triangle*) and tail moment (TM = Tail length × %DNA in tail/100; Olive et al. 1990) as the measure of the extent of DNA damage (TiO_2 catalyst, *black square*; TiO_2–CuO dual catalyst, *gray square*) against time

Killing of other microorganisms

There have been fewer studies on the mechanism of killing of eukaryotes. Linkous et al. (2000) suggested that death of the alga *Oedogonium* sp. was due to nonspecific breakdown of cellular structures. Microscopy has shown membrane damage to the alga *Chroococcus* sp. (Hong et al. 2005). Light microscopy and SEM showed damage to cell walls of *Candida albicans* suspended over a thin film of TiO_2 (Kühn et al. 2003) and on TiO_2-coated tissue conditioner (Akiba et al. 2005). Cell wall and membrane damage to cysts were seen with light microscopy of photocatalytically treated *Giardia lamblia* (Sökmen et al. 2008). Membrane damage was also shown to occur on treatment of the ciliate protozoan *Tetrahymena pyriformis* (Peng et al. 2010).

Killing of *Lactobacillus* phage PL1 by thin films of TiO_2 suspended in liquid was reported to be via initial damage to protein of the capsid by ·OH, followed by damage to the phage DNA inside the particles (Kashige et al. 2001). SEM showed ghost particles and empty heads. Damage to the H and N projections of influenza virus A/H1N1 occurred on PCD and was followed by total mineralisation (Lin et al. 2006).

Spectroscopic studies

The activity of titanium dioxide on isolated phospholipid bilayers has been shown to result in disruption of the bilayer structure using X-ray diffraction (Suwalsky et al. 2005), laser kinetic spectroscopy and attenuated total reflection Fourier transform infrared spectroscopy (FTIR). Disruption was shown to be due to lipid peroxidation (Kiwi and Nadtochenko 2004; Nadtochenko et al. 2006) measured by production of malondialdehyde (MDA). Lipid peroxidation occurs when polyunsaturated fatty acids such as linoleic acid are attacked by ROS (Kiwi and Nadtochenko 2005).

FTIR spectra of treated *E. coli* confirmed the production of carboxylic acids such as MDA as products of membrane degradation. MDA was further degraded by longer irradiation times (Hu et al. 2007).

The electron decay on TiO_2 was studied using laser kinetic spectroscopy in the presence of phosphatidyl ethanolamine, lipopolysaccharide and *E. coli* (Nadtochenko et al. 2006). Spectrosopic studies using FTIR spectroscopy suggested that organic components bound to the TiO_2 were directly oxidised by reduction of the electron holes (Nadtochenko et al. 2006, 2008). This work suggested that direct oxidation of cellular components could occur without the production of ROS, but only if cells were in direct contact with the surface of the TiO_2. This is wholly consistent with the greater effectiveness of PCD when the cells are in contact with the TiO_2 rather than in suspension. Overall, the spectroscopic studies support the light microscopic studies and confirm the order of destruction being OM→IM→PG. Details of kinetic models of the killing mechanism are presented by Dalrymple et al. (2010).

The role of ROS in killing of bacteria is summarised in Fig. 5.

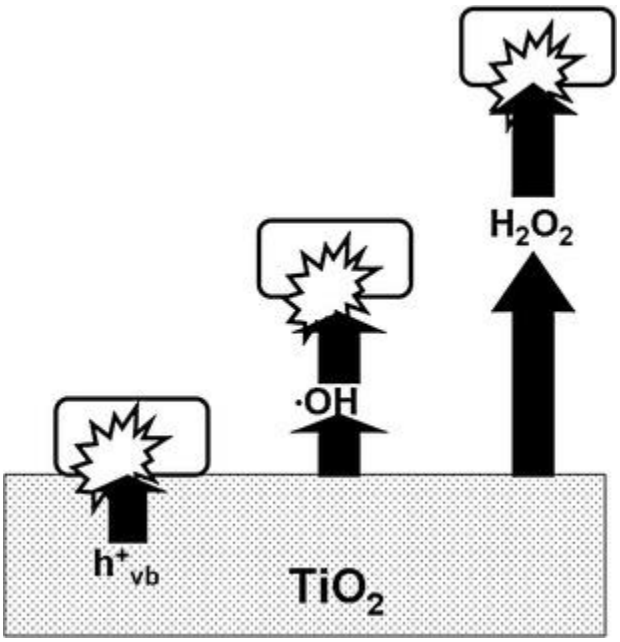

Fig. 5

Role of ROS in photocatalytic killing of bacteria. Direct oxidation of cell components can occur when cells are in direct contact with the catalyst. Hydroxyl radicals and H_2O_2 are involved close to and distant from the catalyst, respectively. Furthermore, ·OH can be generated from reduction of metal ions, e.g. Cu^{2+} by H_2O_2 (Sato and Taya 2006c)

Go to:

Role of ROS in the killing mechanism

Most studies show that ROS are responsible for the killing, and various authors propose that ·OH are responsible (Ireland et al. 1993; Kikuchi et al. 1997; Maness et al. 1999; Salih 2002; Cho et al. 2004, 2005; Cho and Yoon 2008). Lipid peroxidation by ROS was demonstrated by the release of MDA as a breakdown product, and there was a concurrent loss of membrane respiratory activity measured by reduction of 2,3,5-triphenyltetrazolium chloride (Maness et al. 1999). The ·OH scavengers, dimethylsulphoxide and cysteamine, eliminated the PCD activity of suspensions of TiO_2 in water (Salih 2002). However, ·OH are short-lived and will probably not diffuse further than 1 μm from the surface of the TiO_2, especially in the presence of organic matter (Pryor 1986; Kikuchi et al. 1997). Kikuchi et al. (1997) showed that killing of *E. coli* still occurred even when the bacteria were separated from the surface by a 50-μm-thick porous membrane. However, the free radical scavenger mannitol only inhibited killing without the membrane, whereas catalase, which would degrade H_2O_2, decreased killing both with and without the membrane. This suggested that ·OH and H_2O_2 were responsible for killing close to the TiO_2, with H_2O_2 acting at a distance. The role of other ROS, e.g. $O_2^{-}\cdot$ was not considered. However, no killing was seen when cells were separated from the TiO_2 by a dialysis membrane in a separate study (Guillard et al. 2008). Hydrogen peroxide may act at a distance if ferrous ions are present by producing ·OH via the Fenton reaction (8 and 9).

$$Fe^{3+} + O^{2-}\cdot \rightarrow Fe^{2+} + O_2$$
8

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + \cdot OH$$
9

A study of the roles of H_2O_2 and ·OH in an immobilised TiO_2 thin film reactor activated by UVC using electron spin resonance suggested that ·OH were produced by direct photolysis of H_2O_2 as well as by Eqs. 3 and 4 (Yan et al. 2009).

A role for ·OH in sonocatalysis on TiO_2 (where the energy to bridge the band gap is provided by sound waves) was suggested by the work of Ogino et al. 2006 who showed that the killing was inhibited by the ·OH scavenger glutathione. Hydroxyl radicals produced by microwave irradiation of TiO_2 were shown to enhance the killing of *E. coli* (Takashima et al. 2007).

Hydroxyl radicals were shown to be the major ROS involved in killing of *C. parvum* cysts, although other ROS were also involved (Cho and Yoon 2008).

Studies with hydroxyl radical scavengers suggested that inactivation of phage in suspensions of TiO_2 also occurred due to bulk phase ·OH, whereas inactivation of bacteria occurred with both bulk phase and surface ·OH (Cho et al. 2004, 2005). The rate of inactivation of *E. coli* correlated with the concentration of ·OH. A role for other ROS such as H_2O_2 and $O_2^{-}\cdot$ was also suggested.

Studies on superoxide dismutase (SOD)-defective *E. coli* have shown that oxidative damage to the membrane combined with the turgor pressure inside the cell initially permeabilizes the cell envelope, allowing critical metabolites to escape (Imlay and Fridovich 1992). Studies on oxidative damage caused by TiO_2 in SOD mutants of *E. coli* showed that the inactivation rate was inversely proportional to SOD activity (Koizumi et al. 2002; Kim et al. 2004).

Kinetic models and further details of the chemistry of the killing mechanism are presented by Dalrymple et al. (2010). The role of h_{vb}^{+} and ROS in killing of bacteria is summarised in Fig. 5.

Go to:

Importance of contact between bacteria and TiO_2

Many studies have shown that close contact between the bacteria and the TiO_2 increases the extent of oxidative damage. Studies on the disinfection of water have shown that suspended TiO_2 is more active than TiO_2 immobilised on surfaces, e.g. on thin films (Lee et al. 1997; Otaki et al. 2000; Sun et al. 2003; Gumy et al. 2006b; Marugan et al. 2006, 2008; Cohen-Yaniv et al. 2008). This is probably due to increased contact between the TiO_2 particles and the bacterial cells in suspension as well as an increased surface area for ROS production. A number of studies confirm the importance of such contact (Horie et al. 1996a, b, 1998; Gumy et al. 2006a; Pratap Reddy et al. 2008; Caballero et al. 2009; Cheng et al. 2009). Co-precipitation of cells and TiO_2 particles from suspension by alum enhanced killing of *E. coli* (Salih 2004). Certain ionic species have been shown to inhibit PCD, e.g. PO_4^{3-} (Araña et al. 2002; Koizumi and Taya 2002a,b; Christensen et al. 2003; Rincón and Pulgarin 2004b; Egerton et al. 2006; Xiong et al. 2006; Marugan et al. 2008) and HCO_3^{-} (Rincón and Pulgarin 2004b; Coleman et al. 2005; Gogniat et al. 2006), and the rate of adsorption onto the TiO_2 in the presence of different ions correlated with the rate of inactivation, suggesting that the inhibition was due to the prevention of binding of the bacteria to the TiO_2 particles. Light micrographs (Nadtochenko et al. 2005; Gumy et al. 2006b; Gogniat et al. 2006) and electron micrographs clearly show binding of the titania particles to bacterial cells (Gumy et al. 2006a, b; Saito et al. 1992; Cheng et al. 2007; Shah et al. 2008). A micrograph showing particles of TiO_2 attached to an *E. coli* cell is shown in Fig. 6. Contact with highly crystalline TiO_2 may also cause physical damage to the cells (Liu et al. 2007c; Caballero et al. 2009).

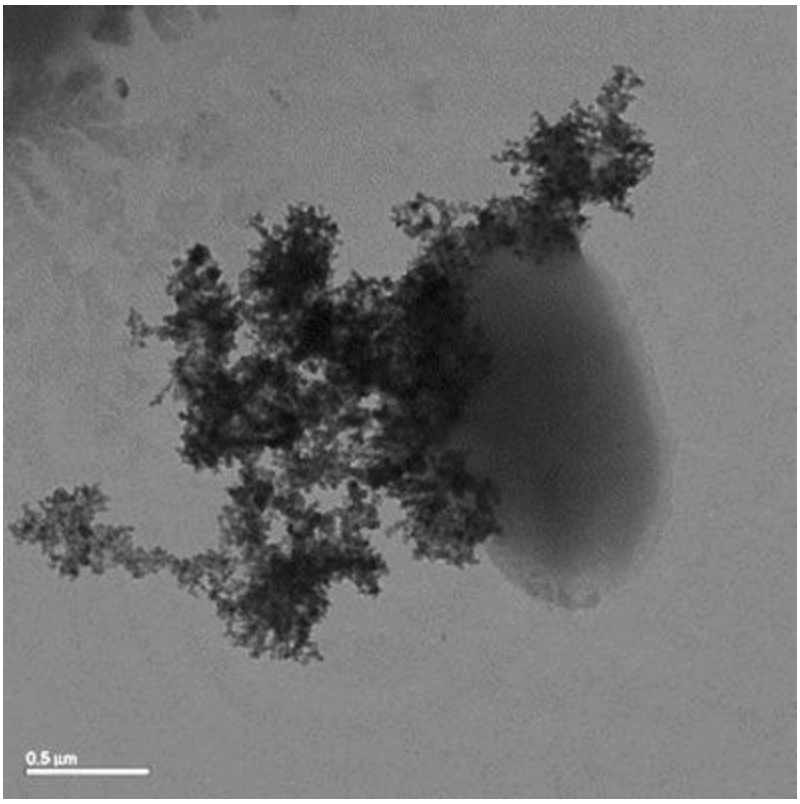

Fig. 6

Transmission electron micrograph of *E. coli* showing adhesion betwen cells and TiO_2 in suspension. Catalyst Degussa P25 pH 6.0. From Gumy et al. (2006b)

Although differences in binding of isolated O antigens to TiO_2 have been shown (*E. coli* O8 and *Citrobacter freundii* O antigens bound strongly to TiO_2, whereas that from *Stenotrophomonas maltophilia* had a low affinity for TiO_2; Jucker et al. 1997), differences in the susceptibility of bacteria with different O antigens have not been studied. Differences in the susceptibility of different strains of *Legionella pneumophila* correlated with the amount of saturated 16C branched chain fatty acids in the membrane (Cheng et al. 2007). The more hydrophobic cells of *Flavobacterium* sp. were more easily killed by PCD than *E. coli* (Cohen-Yaniv et al. 2008), which may also have been due to altered interactions with the TiO_2.

In an attempt to increase contact between the cells, Benabbou et al. (2007) studied the PCD of a strain of *E. coli* overexpressing *curli*, pili, which enhance adhesion to abiotic surfaces. However, the strain was more resistant than the non-piliated control, and evidence of protein degradation suggested that the pili were being degraded before the membrane was damaged and therefore protected the membrane from damage. The presence of extracellular polysaccharides interfered with PCD of biofilms of *P. aeruginosa* (Gage et al. 2005) and a natural biofilm (Liu et al. 2007a), but killing was seen throughout a biofilm of *Staphylococcus epidermidis* on a TiO_2 catalyst (Dunlop et al. 2010). The different biofilms and catalysts may explain these anomalies.

The inhibition of close contact between coliphage MS2 and TiO_2 by certain cations was shown by Koizumi and Taya (2002a, b), and the rate of inactivation was proportional to adsorption of the phage onto the TiO_2. Sato and Taya (2006a, b) showed that the presence of organic materials protected the phage by adsorbing to the surface of the TiO_2, preventing phage binding.

Go to:

Cell mineralisation

Following initial cell damage and cell death, photocatalysis has been shown to be capable of complete mineralisation of bacteria on air filters using ^{14}C-labelled cells (Jacoby et al. 1998; Wolfrum et al. 2002) and for cells suspended in water (Cooper et al. 1997; Sökmen et al. 2001). The total oxidation of *Legionella* by PCO was measured by total organic carbon analysis (Cheng et al. 2007). An almost complete degradation of *E. coli* was demonstrated on prolonged treatment on a TiO_2-activated charcoal catalyst (Li et al. 2008). Nadtochenko et al. (2008) showed total oxidation of cell organic matter by total internal reflection/FTIR. Removal of microorganisms during regeneration of photocatalytic TiO_2-coated air filters by complete removal of contaminants has also been shown by SEM (Goswami et al. 1999; Ortiz López and Jacoby 2002). Penetration of TiO_2 particles into the cells was shown using an $Ag/AgBr/TiO_2$ catalyst (Hu et al. 2006).

A scheme for the killing mechanism of TiO_2 on bacteria is shown in Fig. 7. We suggest that there may be initial damage on contact between the cells and TiO_2 which affects membrane permeability, but is reversible. This is followed by increased damage to all cell wall layers, allowing leakage of small molecules such as ions. Damage at this stage may be irreversible, and this accompanies cell death. As the peptidoglycan is a highly cross-linked molecule, damage may not be visibly evident at this stage or may be localised if the TiO_2 is in contact with the cells. Further membrane damage allows leakage of higher molecular weight components such as proteins. This may be followed by protrusion of the cytoplasmic membrane into the surrounding medium through degraded areas of the peptidoglycan and, eventually, lysis of the cell. Degradation of the internal components of the cell can then occur followed by complete mineralisation.

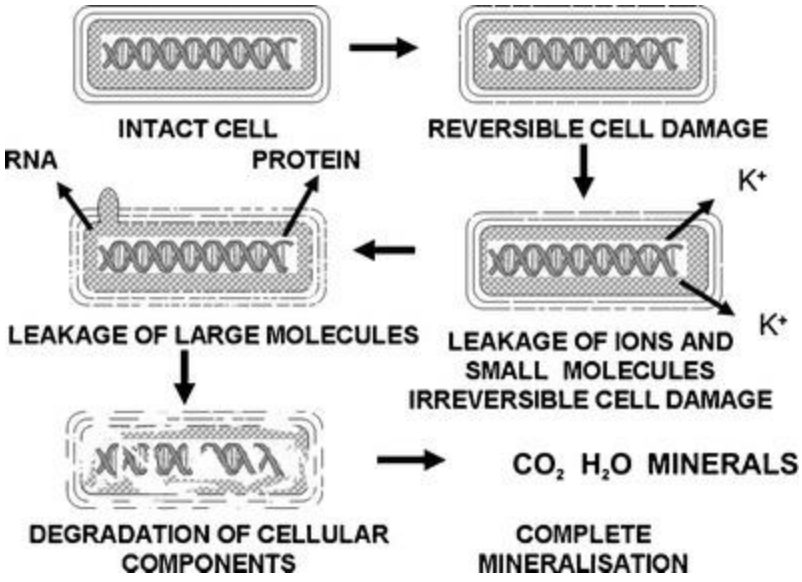

Fig. 7

Scheme for photocatalytic killing and destruction of bacteria on TiO_2. Contact between the cells and TiO_2 may affects membrane permeability, but is reversible. This is followed by increased damage to all cell wall layers, allowing leakage of small molecules such as ions. Damage at this stage may be irreversible, and this accompanies cell death. Furthermore, membrane damage allows leakage of higher molecular weight components such as proteins, which may be followed by protrusion of the cytoplasmic membrane into the surrounding medium through degraded areas of the peptidoglycan and lysis of the cell. Degradation of the internal components of the cell then occurs, followed by complete mineralisation. The degradation process may occur progressively from the side of the cell in contact with the catalyst

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Dual function materials

Copper-deposited films show enhanced PCD activity (Sunada et al. 2003a; Foster et al. 2010; Wu et al. 2010a; Yates et al. 2008a, b). A clear synergy in photokilling of *E. coli* on Cu-containing TiO_2 films was shown by Sato and Taya (2006c) who showed that H_2O_2 was produced from the photocatalyst and Cu^{2+} leached from the surface, but neither reached high enough concentrations to kill the *E. coli* directly. They suggested that the Cu^{2+} was reduced to Cu^{+} (10) which reacted with the H_2O_2 to produce ·OH via a Fenton-type reaction (11), which was responsible for killing cells in suspension and explaining why catalase reduced this activity. Inclusion of Cu also gave higher PC activity, hence the enhanced killing of cells bound to the TiO_2. In our own work, we have seen DNA damage when TiO_2/CuO surfaces were used (Fig. 4). Thus, Cu may also kill cells by DNA damage as well as membrane damage. This is consistent with the observed enhancement of damage to DNA and protein caused by ROS (Cervantes-Cervantes et al. 2005).

$$Cu^{2+} + e^{-}_{cb} \rightarrow Cu^{+}$$
10

$$H_2O_2 + Cu^{+} \rightarrow HO^{-} + \cdot OH + Cu^{2+}$$
11

Similar synergy has been shown between Ag and TiO_2. Ag enhances photocatalysis by enhancing charge separation at the surface of the TiO_2 (Sökmen et al. 2001; He et al. 2002; Hirakawa and Kamat 2005; Kubacka et al. 2008b; Liu et al. 2007b; Musil et al. 2009). Ag^{+} is antimicrobial and can also enhance generation of ROS (Eqs. 12, 13 and 14).

$$Ag^{+} + O_2^{-}\cdot \rightarrow Ag^{0} + O_2$$
12

$$Ag^{0} + O_2^{-}\cdot \rightarrow Ag^{+} + O_2^{2-}$$
13

$$H_2O_2 + Ag^{0} \rightarrow HO^{-} + \cdot OH + Ag^{+}$$
14

Go to:

Conclusions

Generation of ROS by photocatalysis on TiO_2 is capable of killing a wide range of organisms including bacteria endospores in water, in air and on surfaces, including various materials. The technology has the potential to provide a powerful weapon in the fight against transmission of infectious diseases, particularly in view of the development of visible light-activated catalysts.

One of the problems is that until relatively recently, there has not been an accepted standard method for the testing of the antimicrobial efficiency of photocatalytic processes. For example, many different strains of *E. coli* have been used (Table 2) with different growth media and test conditions. This makes it very difficult to compare results from different research groups. In the second part of this review, we will investigate the evaluation of photocatalytic killing activity.

Go to:

Acknowledgements

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