"Transforming Medical Gloves via Active Antimicrobial Technology Using Bespoked Photo-Sensitiser Molecules"

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Abstract

Antimicrobial medical examination gloves have been made cost effectively with non leaching activity. A specialised photosensitiser dye is incorporated into the surface of the nitrile glove. This new molecule uses the energy from light to sensitise oxygen from air to create an energised oxygen molecule, singlet oxygen ($^1O_2$). Singlet oxygen is highly reactive and destroys bacterial cell walls, lipids and proteins. The dye is bonded to the nitrile and cannot be leached out, and the singlet oxygen lifetime is approximately 4 microseconds. These properties give the surface of the glove antimicrobial properties generated from light and air, but without the use of toxic biocides.

Introduction

Healthcare associated infections (HCAI) have a prevalence of approximately 5-10% of all hospital admissions. They also occur in community practices, longer term care facilities and other healthcare providing facilities. They are extremely costly in both human terms and costs to health care providers. In the US, there were 722,000 patients with a HCAI in 2011, and 75,000 deaths, with associated direct costs of between $28-$45Billion. In the EU, HCAI infected 4.1 million patients per year in 2013, with 37,000 deaths and direct costs of >7Billion Euro. Literature data shows patients with a HCAI cost three times as much to treat as patients with no HCAI, and their stay in hospital is three times as long. Treatment for these infections is also becoming more difficult with increasing numbers of bacteria developing resistance to known antibiotics[1-6]

It is known that the primary mode of infection (up to 80%) involves transfer during procedures being done by healthcare workers – from hands. Up to now, disposable medical examination gloves have proven a useful barrier, but a passive one. Once bacteria are on examination gloves it is known that they can be transferred off to patients and the local environment[7-9]. An active antimicrobial examination glove would provide additional protection to break the primary route of transfer for HCAI – via healthcare workers hands.

Development – Approach

At the outset of the project we set ourselves several key challenges. Most important was that any antimicrobial technology should be non leaching. We wanted no antimicrobial materials to leach from the gloves, to design in safety from the start.

We also knew that to make the product make a difference in real healthcare settings that it should be a universally usable glove, without significant additional costs. In turn this meant that the current manufacturing process would have to provide the completed antimicrobial glove, with minimal modifications or no offline processing.

The final key challenge was speed of kill of microbes, and efficacy. Many antimicrobial systems can take long contact times to achieve a good kill. Medical examination gloves however are only worn on average for approximately 10 minutes. Some are worn longer, some shorter. To break this route of transfer the kill would have to be very rapid, preferably minutes.

Existing technologies

We reviewed the commonly used approaches to antimicrobial kill at the outset. Traditional biocides are widely used and incorporate such materials as PHMB (polyhexamethylbiguanide), Chlorhexidine, Quaternary ammonium compounds. The disadvantage of these approaches was complexity in incorporating them into a glove, leaching, toxicity and cost. Silver antimicrobials are of increasing interest. In these systems a silver ion (Ag+) source is included into the material, and silver ions slowly leach out creating an antimicrobial effect. The main disadvantages of silver technology however are that it is almost always slow to achieve kill; it is also essentially still a leaching technology. We were also somewhat concerned by the potential for resistance to develop to silver, as several literature reports had noted this occurring[10-12]

Technology Choice - Background

After reviewing, we chose to use a novel method of achieving efficacy that has not to date received as much attention. This is singlet oxygen based. In this technology a special dye is used. The dye absorbs light, usually available visible light. The dye is thus in an excited quantum state. The energy is then transferred to a proximal oxygen molecule, and the oxygen molecule is raised to an excited quantum state. The ground state of oxygen, as present in air, is triplet electronic configuration, written as $^3O_2$. On sensitisation by the dye molecule the electronic configuration is changed, and it enters the singlet state $^1O_2$.

This singlet oxygen state is reactive, and more oxidative than ground state oxygen and is able to kill microbes. It is also quite unstable however, and has a lifetime of around 4 microseconds, giving it an estimated diffusion distance of ~150nm[13]. Note that as soon as the dye passes on its energy to an oxygen molecule it reverts to its ground state and is available to absorb another photon and excite another oxygen molecule. The dye is therefore a catalyst in this system and can continually generate more singlet oxygen. The killing species is the short-lived singlet oxygen, and is generated from light and air, using the dye as a catalyst.

Whilst it has not received as much attention as traditional biocides, singlet oxygen has been researched for a wide range of uses for many years and a number of important commercial applications are known[14]. In humans, singlet oxygen generating dyes are used for cancer treatment, known as photodynamic therapy, PDT. It is also used in dental disinfection prior to procedures like root canal treatments, in which the dye is rinsed into the patients mouth, a light applied and disinfection occurs safely and rapidly. However probably the most ubiquitous use is in laundry powders, where a singlet oxygen generating dye is washed onto clothing, and subsequently acts as a photobleach. Many readers of this will therefore be unwitting users of singlet oxygen and will be wearing some singlet oxygen generating dye.

Technology Choice – Practical Application

We naturally screened the available singlet oxygen generators, but after much work we found them all deficient in one aspect or another. For our process, a specific set of requirements are present. For example, the singlet oxygen generating dye (SOG) must be water soluble in coagulant, containing 10-20% calcium nitrate, as well as mould release and other agents. It must be thermally very stable at high temperatures over long periods, and in some parts of the process very high temperatures for shorter times. It must also be stable to the environment of the nitrile during vulcanisation where there are organic radicals present, as well as many other reactive species. A final, but key requirement for our application is zero leachability of the dye.

We therefore designed our own bespoke molecule to fit in with these requirements. Our designed molecule has features that bond to the nitrile. It is also thermally stable, soluble in coagulant solution, and compatible with all the materials present in the process, and economical to produce at scale, as well as providing the necessary efficacy.

Multiple production trials have now been run with this technology included.

Results and Discussion

There are many potential test methods for determining efficacy of antimicrobial materials. We have chosen to principally use a published test method ASTM D7907 - Determination of Bactericidal Efficacy on the Surface of Medical Examination Gloves, because this is a published test method allowing others to repeat the tests, and it is specifically designed for this application. All our tests are done by FDA accredited independent 3rd party labs. The Table below illustrates some typical data on our gloves using this test method.

		Average % Kill			
Microbe	Type	5 mins	10 mins	15 mins	20 mins
Enterococcus faecalis (VRE)	Gram positive	99.982%	99.996%		99.968%
Enterococcus faecium	Gram positive	99.991%	99.991%	99.996%	
MSRA	Gram positive	99.988%	99.998%	99.999%	99.997%
Staphylococcus aureus	Gram positive	99.996%	99.993%		99.994%
Streptococcus pyogenes	Gram positive	99.946%	99.970%	99.988%	99.996%
Escheri coli	Gram negative			99.030%	
Klebsiella pneumoniae	Gram negative		96.471%		97.747%

There gloves kill rapidly and to very high levels for a wide range of organisms of clinical importance. The antibiotic resistant strains, such as MRSA and Vancomycin Resistant Enterococcus are just as effectively killed. This is due to the mechanism of killing. Singlet oxygen is a strong oxidiser, it attacks many different sites on the bacteria (antibiotics, because the are used in humans have to target much more carefully and use a specific lock and key type mechanism)[15].

There are a few other important points to ~~no~~te about the microbiology. Firstly, complete kill is not required. In infection there is a concept of "infectious dose". This is a count of the number of organisms required to create an infection in a patient. It varies very widely with organism, but also with circumstances, for example patient immune capability, and site of transfer/infection. Numbers range from counts of single digits, up to 10^4 organisms before an infection is possible. Therefore, if we successfully reduce the load of the organisms from say 500 to 50, this is a 90% kill, but may still reduce the rate of HCAI[16-18].

We can note somewhat slower kill for gram negative type organisms in this data set. This is not completely surprising, many traditional biocides also kill gram negative organisms more slowly, or less completely[19,20]. This is due to the different nature of the cell wall. Gram positive organisms have a generally more porous cell wall, gram negative cell walls are more complex, so these organisms are often more resilient to biocides. However, we can also note that gram negative organisms also die more quickly on surfaces, sometimes within a few minutes to hours, so may not be as available for the hand transfer route to create infections[21,22]. Gram positive organisms are known to survive relatively longer on surfaces.

Although the shortest test time provided by ASTM D7907 is 5 minutes, we decided to look at shorter times because we think transfer from surface to surface can happen very rapidly and may be important in HCAI. The table below shows the results for a selection of organisms at 1, 2 and 5 minutes.

% Kill/Organism	1 minute	2 minutes	5 minutes

Staph aureus	99.990%	99.998%	>99.999%
Enterococcus faecalis (VRE)	>99.998%	>99.998%	>99.998%
Streptococcus pyogenes	>99.998%	>99.998%	>99.998%

One other important parameter for antimicrobial testing is soil conditions. These are intended to simulated conditions where the gloves are non only contaminated by microbes but also other material, such as sweat, saliva, mucus or blood. Of course we would not recommend wearing contaminated gloves, and these should be changed as soon as any contamination is visible before engaging any further procedures. We have therefore selected light to moderate soil conditions that may not always be visible. These are taken from the literature and from published standards[23,24]. These gloves do retain good activity even under soiled conditions.

		Average % Kill		
Microbe	Type	5 mins	10 mins	20 mins
Enterococcus faecalis (VRE)	Gram positive	99.968%	99.995%	99.995%
Staphylococcus aureus	Gram positive	99.776%	99.917%	99.922%
Klebsiella pneumoniae	Gram negative	36.904%	97.049%	96.198%

Further data on the killing properties of these gloves are being collected.

Biocompatibility

Extensive leach testing using extractants such as water, hot water, ethanol, simulated saliva and simulated sweat has demonstrated that the dye is effectively bound to the latex and cannot be leached out.

Standard biocompatibility testing using ISO 10993, both inner and outer surfaces shows the gloves to not have skin irritation or skin sensitisation properties. The extracts are not cytotoxic, and there is also no oral toxicity.

The Modified Draize-95 test was also conducted where the inner and outer surfaces of the gloves were tested on human skin. No clinical evidence to demonstrate that this glove may induce allergenicity.

All the other physical properties of the nitrile glove remained unchanged.

Potential for resistance

It is important to consider the potential for organisms to develop resistance when using an antimicrobial system[29]. The advantage of singlet oxygen is that is a non selective system, and reacts rapidly with many microbial components. There is therefore not one single mechanism of protection that a bacteria for example could

use to protect itself from singlet oxygen – unlike in antibiotics which need to use a very specific mechanism[25].

Some mechanisms that bacteria use for dealing with other reactive oxygen species are known, for example the enzyme superoxide dismutase can effectively quench superoxide anions. However there are no known mechanisms for protecting against singlet oxygen, and in fact singlet oxygen is known to destroy superoxide dismutase. Many of the mechanisms bacteria use to confer resistance involve processes internal to the cell. In our system however, the singlet oxygen is generated purely exogenously to the cell, because the dye is separated from the bacteria, and cannot enter the cells. Other authors in the literature have noted that this makes development of resistance especially difficult, because singlet oxygen is short lived and with a short length of diffusion – nothing the bacterial cell does internally will affect the process of oxidation by singlet oxygen.

Several literature reviews have been published that evaluate the potential of resistance developing to singlet oxygen, and all authors conclude that the possibility is very low, because of the non specific way singlet oxygen reacts, and that it has such a short lifetime that extended exposure to sub-lethal doses is not possible[25, 26].

In addition experimental studies have been done and reported in the literature[27,28]. In these, bacteria were killed to a high extent, typically 99.9% or 99.99%, leaving only the most robust bacteria. These were then re-cultivated and re-exposed to singlet oxygen. This cycle is repeated 10 or 20 times, and the efficacy of killing is measured. In all cases, it was found that no decrease in efficacy was seen, and no resistance developed.

Summary and Conclusions

The antimicrobials gloves we have developed use a novel approach to killing microbes in order to deliver cost, safety and speed of kill. The dye catalyst needed to be specifically designed to fit into a glove manufacturing process and to bond to the nitrile to enable non leaching.

There are many approaches to intervening in HCAI, but many of these require changes to procedures in already stretched health care systems, additional capital, ongoing costs etc. The antimicrobial examination gloves do not require any additional costs or changes and are the same as standard nitrile examination gloves in all other respects. They are simple for healthcare workers to use and should prevent the cycle of transfer and infection that is currently responsible for so many diseases, deaths and costs in healthare.

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