2019

Anti-Viral Abilities of DABCO-Conjugated Molecules

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An Honors College Thesis

by
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Spring, 2019

Biology

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Date

Table of Contents

Abstract

Viral infections cause many detrimental diseases throughout the world and are difficult to treat due to their use of the host's own cells. In addition, viruses have high mutation rates making them difficult to target with medical treatments. Studies done with DABCO-Conjugated molecules have showed promising results in removing viruses from environments. DABCO molecules are unique due to their ability to bind covalently to carbohydrate and protein based surfaces, such as cotton and wood. This provides opportunity to create antiviral surfaces, such as filters and fabrics which can be used to treat viral infections and minimize the possible transfers. It is proposed that a certain DABCO-conjugated molecule, diDABCO-C4, can bind viruses.

Background

Viral infections are commonly seen in humans and no effective treatment is available. Many different viruses capable of infecting humans exists. Some viruses, such as the rhinovirus can cause a common cold whereas others, such as HIV, cause the chronic and fatal disease AIDS. There is currently 36.9 million people living with HIV and although treatments exist, they are not able to cure the disease. The same is true for many other chronic viral diseases such as hepatitis. Viral infections are hard to treat due to them existing and residing within the host's cells. Furthermore, many have high mutation rates, which often makes available treatments less effective over time. It is possible that some of these viruses could become less of worrisome if an effective treatment was discovered. The DABCO-conjugated molecule has showed promising results in respect to its antimicrobial effects. Significant results have been recorded with bacteria, fungi, and viruses. A DABCO-conjugated molecule could change the way viral infections are treated by creating blood filters as well as minimize the spread of viruses using DABCO treated air filters, uniforms, and surfaces.

Virus

A virus is an obligate parasite of animals, plants, and bacteria. It needs a living host for reproduction and due to the great variety of virus structure and function a wide range of hosts exists. A virus's genome can be found inside a capsid made of either protein or proteolipid. This capsid protects the genome when transferred to the host. On the capsid there is also host recognition sensors which help the virus identify the host cell and is used as an anchor for transmission of the virus genome. Two types of viruses exist, enveloped and non-enveloped. Enveloped viruses have a lipid bilayer with viral proteins embedded giving it a spikey look. The envelope surrounds the capsid and the proteins embedded in the membrane are used for host

recognition, attachment, and entry into the host cell. Non-enveloped viruses do not have a lipid bilayer and its outermost layer is the capsid.

Bacteriophages are a type of virus that infects bacterial cells. Bacteriophages ranges in their complexity from simple spherical viruses with genomes of 5 kbp to complex viruses with genomes of more than 280 kbp (Leiman, Kanamaru, Mesanzhinov, Arisaka, & Rossmann, 2003). The majority of bacteriophages have a special molecular device called a tail, which is used for recognition and attachment to a host cell, penetration of the cell envelope, and for DNA or RNA transfer from the virus capsid into the host cells cytoplasm (Leiman, Chipman, Kostyuchenko, Mesyanzhinov, & Rossmann, 2004).

Bacteriophage T4 (Figure 1)

There are seven bacteriophages that can infect *E. coli*; the three "even" phages – T2, T4, and T6 – have similar structure, antigenic response and genetics (Yap & Rossmann, 2014). Bacteriophage T4 has double-stranded DNA and is one of the most complex viruses, with a genome containing 274 open reading frames out of which more than 40 encode for structural proteins (Leiman, Kanamaru, Mesanzhinov, Arisaka, & Rossmann, 2003). Bacteriophage T4 is a member of the Myoviridae family, which is in the order *Caudovirales* due to its contractile tail (Yap & Rossmann, 2014). It consists of a 5-fold-symmetric head containing the 172 kpb genomic DNA, a 6-fold-symmetric bilayered tail, and six long fibers attached to the tail's baseplate (Leiman, Chipman, Kostyuchenko, Mesyanzhinov, & Rossmann, 2004). Bacteriophage T4 initially recognize the host via a reversible interaction of the tips of the long tail fibers with lipopolysaccharide cell surface receptors. The baseplate has a hexagonal shape in the mature virus, but after adsorption to the host cell it changes into a star shape. It has been proven more than once that the bacteriophage corpuscle bears a negative charge over a range of H-ion concentration from pH 3.6 to 7.6 (Krueger & Ritter, 1929).

Quaternary ammonium compounds (QUATs)

Quaternary ammonium compounds have shown antimicrobial abilities, they are positively charged surfactants due to four different R-groups being attached to the nitrogen atom (**Figure 2**). The R-groups can be alkyl or aryl groups of different lengths. The length of these groups has been shown to play a role in the antimicrobial activity of QUATs. Previous research has shown that QUATs with a hydrocarbon chain of 16 carbons affected the outer membrane of Gram-negative bacteria more than shorter hydrocarbon chains. It was proposed that this was due to an interaction between the hydrophobic C_{16} chain and the fatty acid portion of lipid A, which is found in the membrane (Ioannou, Hanlon, & Denyer, 2007). This interaction disrupts the membrane and cause cell leakage, resulting in lysis and cell death.

Early studies have shown that cationic surfaces had antiviral effects. Armstrong and Froelich worked with benzalkonium chloride which is a cationic surface. They found that ether-sensitive viruses such as arbo, herpes, and myxo related viruses, were sensitive to benzalkonium chloride as well as a couple of ether-resistant viruses, poxviruses and adenovirus (Armstrong & Froelich, 1964).

Huang investigated the adsorption of the Influenza virus to charged surfaces on both natural and artificial surfaces. He suggested that the interaction between neuraminic acid could be due to electrostatic interactions rather than structure of a surface receptor. He proposed that if that was the case then one should be able to attract viruses with other charged molecules. His results showed support of his hypothesis (Huang, 1974).

DABCO Molecule

1,4-diazabicyclo [2.2.2] octane (DABCO) is similar to QUATs but contains two nitrogen molecules in an aromatic bicyclic structure (**Figure 3A**). In addition, it can be covalently bound to carbohydrates and protein based surfaces, which makes them unique (Engel et al., 2009). The

molecule can be manipulated to include more DABCO rings or hydrocarbon chains of different lengths (Engel et al., 2009). The charge of the molecule depends on the number of DABCO molecules and structures attached to the molecule, each DABCO bringing additional positive charges. By modifying the molecule and its attached hydrocarbons, the molecule can display a variety of antimicrobial abilities. Earlier investigations have shown that the mode of action is both a physical disruption and an electrostatic interaction of the membrane as seen in **Figure 4** (Abel, et al., 2002). The DABCO-conjugated molecule has a positive charge attracting the negative charge of the bacterial cell wall in an electrostatic interaction. Upon contact, the conjugated hydrocarbon molecule physically disrupt the cell wall by piercing it. A DABCO molecule covalently bound with a hydrocarbon of 16 carbon molecules attached to cotton cloth had antibacterial activity against *E. coli*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Proteus vulgaris*, *Bacillus cereus*, *Micrococcus luteus,* and *Staphylococcus aureus* (Abel et al., 2002). The positively charged DABCO portion of the molecule attracts the negatively charged lipopolysaccharides in the Gram-negative and techoic acids in the Gram-positive bacterium. The structure of the DABCO-Carbon 16 molecule (**Figure 3B**) allows the hydrocarbon chain to pierce the bacterial cell wall as the bacterium approaches and disrupt the membrane, ultimately resulting in lysis and death (**Figure 4**). In contrast, the diDABCO-C4 molecule (**Figure 3C**) does not have antibacterial effects due to the hydrocarbon chain being in between two DABCO molecules making it impossible for it to pierce and destroy the bacterium.

Previous research done in the Melkonian laboratory, by master student Elizabeth Stirling, have shown the diDABCO-C4 molecule have antiviral effects possibly due to electrostatic interactions. Experiments with T4 bacteriophage and adenovirus showed a possible inactivation of viruses, whereas results from working with the Influenza virus (H_3N_2) were inconclusive. The

research done with the non-enveloped viruses (T4 bacteriophage and adenovirus) were based on an electrostatic interaction. The experiment attempted to break this interaction using two types of detergents, SDS and Triton X-100. SDS were effective at releasing bound virus from the diDABCO-C4 molecule whereas Trition X-100 was not. An SDS-PAGE was also run in order to identify possible viral protein particles in SDS detergent solution after stripping. These results were inconclusive.

Detergents

The DABCO-conjugated molecules are positively charged attracting the negative charge found in bacterial, fungal, and viral structures. Detergents are negatively charged and would therefore be attracted to the DABCO molecule rendering it unable to interact with microbes. If detergent can inactivate the DABCO-conjugated molecules, detergent could be used to release bound virus, which could thereafter be detected by an antibody assay or SDS-PAGE.

Detergents are often used to denature proteins before running an electrophoresis but can also be used to break interactions between molecules and viruses. Many different detergents exist; some detergents are ionic while others are non-ionic. Ionic detergents are stronger and able to break non-covalent bonds, whereas non-ionic detergents are milder. Sodium dodecyl sulfate (SDS) is an ionic detergent used to denature proteins by binding to amino acids, one detergent molecule for every two amino acids, which overwhelms the protein with negative charge causing it to unfold. It has a polar end and a non-polar end where the hydrocarbon tail is found. Triton X-100 is a nonionic detergent containing a hydrophilic polyethylene oxide chain and a hydrophobic aromatic hydrocarbon group. Triton X-100 is a milder detergent than SDS and is used to disrupt protein-protein interactions.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Electrophoresis can be used to detect proteins of different sizes, even viral proteins in a solution. This technique can be utilized in this research to detect whether viral proteins are present in detergent solution after DABCO treated cloth has been washed. SDS-PAGE separates macromolecules in an electric field; a common type of electrophoresis for protein separation is SDS-PAGE. It uses a polyacrylamide gel as the supporting medium and SDS detergent to denature proteins. When a protein is treated with SDS its polypeptide chain binds it and the amount of SDS bound to the chain is in proportion to its relative molecular mass. The negative charge that SDS carries destroys the structure of the protein and the charge is attracted toward an anode when in an electrical field. The polyacrylamide gel restricts larger molecules from migrating as far and fast as smaller molecules. The final separation of proteins is dependent almost entirely on the differences in the molecular mass of the polypeptide because the charge-to-mass ratio is nearly the same among polypeptides denatured with SDS.

When running a SDS-PAGE of an unknown, proteins of known mass are run simultaneously so that the distance and mass can be correlated and plotted in order to determine the unknown. The molecular weight is expressed in Daltons, where one Dalton is defined as 1/12 the mass of carbon 12. Since most macromolecules are large, kilodalton (kDa) are usually used when describing molecular mass.

SDS-PAGE can separate proteins relative to their mass, relative abundance of major proteins in a sample, or to determine the distributed of proteins among fractions. Different staining techniques can help visualize rare proteins and give information about biochemical properties. Western blotting is one type of technique that can be used to detect extremely scare gene products, to find similarities and detect and separate isoenzymes of proteins. Another

technique is silver staining; proteins binds silver ions, which can then be reduced and thereby visualized.

Thesis Goals

Based on previous findings by Master's student Elizabeth Stirling in the Melkonian lab, this thesis will examine the mode of action of the diDABCO-C4 molecule on T4 bacteriophages. T4 bacteriophages was chosen because they are easy to work with and safer than the human pathogens, adenovirus and influenza virus. Also, their host, *E.* coli, is readily available for use. Based on the proposed electrostatic interaction, where diDABCO-C4 works similar to a magnet attracting viruses, this study will attempt to determine if the virus is temporarily inactivated while bound to diDABCO-C4, but still infective once removed, or if the virus is permanently inactivated and not capable of infection upon release. This is of interest if the technology is to be used in the medical field and help stop the spread or treat viral infections. Because it has been suggested that the interaction is electrostatic, different detergents will be used in an attempt to strip virus off the cloth and determine its infectivity. In addition, a SDS-PAGE will be run to determine the presence of viral proteins after detergent treatment.

Materials and Methods

Determining optimal concentration of T4 bacteriophage

Tenfold serial dilution of T4 virus was prepared in SM+G (5.8 g of NaCl, 2 g of $MgSO_4$: $7H_2O$, 50 ml 1 M Tris-Cl (7.5 pH), 2% (w/v) gelatin solution in 1 liter of dH2O) in microtubes. 100 µl of each dilution was transferred into new microtubes containing 100 µl of *E. coli*. The *E. coli* virus mixture was then placed in a 37°C incubator for 20 minutes. Three ml of top agar (7 g/liter of Bacto agar) pippeted into 14 ml Falcon snap cap tubes. 100 µl of the *E. coli* virus mixture was added to the top agar and swirled. The soft agar was poured onto a TSA (tryptic soy

agar) plate. The plates were incubated 37°C overnight, and the resulting viral plaques were counted manually.

Evaluation of DABCO treated cloths

Virus was diluted to 10^{-5} with SM+G buffer. Four DABCO treated cotton cloths, one DABCO-C16 and 3 different diDABCO-C4 as well as two blank cloths (untreated/control) were cut into 1.5cmx1.5cm squares and placed into individual microfuge tubes. The 1ml of the 10^{-5} virus dilutions was pipetted into each microfuge tube. Two additional control tubes were prepared, one containing the virus dilution only and another containing only SM+G. The tubes were then incubated in a cold room (4°C) overnight on a rotator. For each microfuge tube, a new corresponding microfuge tube was labeled and 100 µl of *E. coli* was added, followed by 100 µl of the corresponding viral solution. Tubes were incubated for 20 minutes at 37 °C in an incubator before being plated and incubated 37°C in an incubator overnight. The plaques were counted manually the following day.

Detergent effect on E. coli

900 µl of SM was added to five microfuge tubes. In tube one, we added 100 µl of 10% TX-100, in tube two 100 µl of 20% NaCl, in tube three 100 µl of 20% SDS, and in tube four 100 µl of 5% SDS. Next, 100 µl of each solution was transferred into new microtubes containing 100 µl of *E. coli* and incubated for 20 minutes at 37°C. 100 µl of each tube was added to three ml of toft agar in a snap cap tube. The tube was swirled and poured over a TSA plate. TSA plates were incubated overnight. Plaques were counted the next day.

Detergent effect on T4 bacteriophage

We added 1 ml of 10^{-4} diluted virus into 4 microtubes. We then added 100 µl of 10% TX-100 into one, 100 µl of 20% NaCl into two, and 100 µl of 5% SDS into three. The last microtube served as a control. The microtubes were then rotated for 50 minutes at room temperature. Next,

we added 100 µl of each tube was transferred into new microtubes with 100 µl of E. coli and incubated the new tubes for 20 minutes at 37°C. After incubation,100 µl of the mixture was added to three ml of top agar and poured onto TSA plates. Plates were incubated overnight at 37°C and the number of plaques were counted the next day.

Inoculation and stripping of cloth

We divided 30 microfuge tubes into six groups of five. The first three groups of five was filled with 1 ml of virus diluted to 10^{-1} in SM. The other three groups were filled with 900 µl of SM. We cut 1.5cmx1.5cm pieces of cloth treated with di-DABCO-C4 and placed one in each microtube before rotating them for 2 hours at room temperature (22°C). Once the inoculation had finished, cloths were washed in a conical tube twice with SM by rotating end-over-end. Six new microfuge tubes were labeled. Microfuge tube one and four contained one ml of 5% SDS, two and five had one ml of 20% NaCl and three and six had one ml of SM (control). One cloth at a time from group one was transferred into microfuge tube 1 and rocked for 5 minutes at 22°C. Cloths were then removed and placed into individual 50ml conical tubes containing 2 ml of SM. Lastly, 100 µl of microfuge tube solution was transferred into new tubes containing 100 µl of SDS-PAGE sample buffer.

Gel Electrophoresis

The 6 microfuge tubes from the inoculation and stripping of cloth section was used as samples to run for the SDS-PAGE. A Mini-PROTEAN® TGX Stain-Free™ Gel by BIORAD was used. Lanes two and ten were loaded with 20 µL Prestained SDS-PAGE standards. Between samples a blank lane was kept in order to prevent bleeding of the samples. Lane four contained a 10^{-1} viral dilution as a positive control. Lane six contained a negative control with only cloth and detergent present and lane eight was loaded with a solution of virus stripped with SDS after inoculation with a diDABCO-C4 cloth.

The gel was run at 150 volts for 1 hour in 1x running buffer (120 g Trizma Base, 576 g glycine, 40 g SDS brought to 4L dH_20). The gel was stained with silver to examine the presence or absence of virus according to Chevallet et al. methods (Chevallet & al., 2007).

The gel was placed in a 50% methanol solution for 1 hour at 23°C with rocking. The gel was added to a silver-ammonia solution (42 ml 0.36% NaOH, 2.8 ml ammonium hydroxide and 1.6 g $AgNO_3$ in 8 ml dH_2O) for 15 minutes at 23°C with rocking. The gel was then washed five times with dH_2O for two minutes each at 23°C with rocking. To develop the gel, it was placed in developing solution (2.5 ml 1% citric acid and 0.25 ml 25% formaldehyde and brought to 500 ml with dH_2O) for 10-20 minutes at 23°C. The gel was then placed in 50 ml Stop Solution (225 mL methanol and 5 mL acetic acid and brought to 500 mL with dH_2O) to inhibit further development.

Results

Determining optimal concentration of T4 bacteriophage

We were interested in finding a virus dilution that would result in 25-250 plaques for all experiments. After running a viral plaque assay, the 10^{-5} dilution plate was found to have 322 plaques. Plates 10^{0}-10^{-4} had too many plaques to count and plate 10^{-6} had 27 plaques.

Evaluation of DABCO treated cloths

Results from the cloth treatments is shown in **Table 1** and images for A, B and AB blank can be seen in **Figure 6**. They indicated that cloth B was most effective at inactivating virus. The JC1, JC2, and A cloth viral plaque counts were all greater than their matching blank cloths. Cloth B showed a 46% decrease in viral plaques. Cloth C had a 28% decrease, cloth D a 42% decrease, cloth E 37% decrease, and cloth F 18% decrease. All cloths were treated with diDABCO-C4, except JC1 which was treated with DABCO-C16.

	NUMBER OF PLAQUES	PERCENT DIFFERENCE FROM BLANK

JC1	TMC	-
JC2	313	11% increase
JCL BLANK	283	-
A	322	13% increase
B	152	46% reduction
AB BLANK	284	-
VIRUS ONLY	TMC	-
SM+G	0	-
C	257	28% reduction
D	207	42% reduction
E	223	37% reduction
F	292	18% reduction
CDEFG BLANK	355	-

Table 1. Number of plaques found on plates after cloth treatment and percent difference form corresponding blank/untreated cloth.

Detergent effect on *E. coli*

To ensure that the detergents to be used in this experiment did not have detrimental effects on the virus' host cell (*E. coli*), bacteria were treated with various detergents and plated as described in Methods. All detergents treatments resulted in a full lawn of *E. coli* as observed for the *E. coli* only control. Therefore, the detergents tested did not interfere with the growth of *E. coli.*

Detergent effect on T4 bacteriophage

SDS is a strong detergent, so in order to ensure the virus would not be destroyed by the detergent it was examined as the method sections states. SDS could possible denature the viral capsid and viral protein, which would affect the results, in particularly, the Gel electrophoresis results. The results are shown in **Table** 2, the control plate had 283 viral plaques present, the plate treated with 10% TX-100 had 304 viral plaques, the plate treated with 20% NaCl had 235 viral plaques, and lastly the plate treated with 5% SDS had 195 viral plaques.

	NUMBER OF PLAQUES	PERCENT DIFFERENCE
VIRUS ONLY	283	-

10% TX-100	304	7% increase
20% NACL	235	17% reduction
5% SDS	195	31% reduction

Table 2. Number of viral plaques after detergent treatment of T4 and percent difference from control plate.

Gel Electrophoresis

A gel electrophoresis was of interest because it would show the presence of viral proteins in a solution. Once the cloth had been treated with detergent it would be expected that the virus was no longer interacting with the diDABCO-C4 molecule attached to the cloth but was instead present in the solution with the detergent. A comparison between a positive control and detergent solutions were attempted but, unfortunately, the silver staining procedure did not stain the gel and therefore no conclusion can be made.

Discussion

Evaluation of DABCO treated cloths

We expected that all diDABCO-C4 cloths - JC2, A, B, C, D, E, F - would have reduced the viral load after inoculation based on an increased positive charge in comparison to DABCO-C16 and previous results (Stirling, 2017). Cloth B, C, D, E, F all showed reductions whereas A and JC2 did not. We did not expect JC1 to interact with the virus since it was modified with the DABCO-C16 molecule, which carries less charge due to only having one DABCO molecule resulting in a net charge of 2+ (**Figure 3B**). The diDABCO-C4 molecule contains two DABCO molecules resulting in a net charge of 3+ (**Figure 3C**), making it more positive and more likely to attract the negatively charged T4 bacteriophage.

Early work done by Master's student Elizabeth Stirling showed reductions greater than 90%. The percent reductions seen were lower than expected and might indicate that the molecule

attached to the cloth might degrade over time since the cloths were not newly synthesized. We were not able to work with newly synthesized cloth before the thesis was due, but it would be of interest to compare a newly synthesized cloth with an older cloth to see if there is a difference in antiviral activity and to determine how long the treatment lasts.

Detergent effect on T4 bacteriophage

We ran this experiment because we wanted to make sure the virus would not be destroyed when treated with 20% NaCl and 5% SDS. We did not expect Triton X-100 to damage the virus due to it being a non-ionic detergent. The results showed that SDS did lower the number of viral plaques, however, it was not a significant decrease and still resulted in 195 viral plaques, which fell into our optimal range of 25-250 plaques. Based on the results we decided to use 20% NaCl and 5% SDS for the inoculation and stripping of cloths. We decided to not use Triton X-100 because we were not sure if it would be strong enough to break the interaction between the virus and diDABCO-C4 molecule.

Gel Electrophoresis

Problems staining the gel after running the gel electrophoresis were encountered. Therefore, no bands were observed, and interpretation of results were not possible. It would have been expected that the gel would have stained as seen in **Figure 7**. If there had been more time, we would have liked to use antibody detection due to its specificity as well as run another SDS-PAGE gel and silver stain.

It would have been expected that lane eight would have been similar to lane four because viral proteins were expected to be present in the detergent solution. Lanes two and ten, the ladders, were used for reference. Lane six were not expected to show any bands since it did not contain any virus and functioned as a negative control.

Examining the silver stained gel in **Figure 7.** it can be observed that the bands in 4 is not seen in lane 8 where they were expected. This could possibly indicate that the SDS detergent did not strip the cloth well, leaving little to no virus in the solution, that the virus was never bound to the cloth, or that the SDS destroyed the viral proteins. Another possible reason as to why no virus was detected is that the interaction between diDABCO-C4 and the bacteriophage is not electrostatic. If that is the case, the SDS would not have broken the interaction and the virus would still be attached to the cloth.

Future Experiments

Future experimentation is needed to obtain significant results. Multiple SDS-PAGE tests would be needed to confirm removal of any bound viral particles. Based on those results an antibody assay would be of interest to determine that the proteins are in fact the viral proteins of interest. Lastly, once virus has been removed from DABCO treated cloth a plaque assay would be performed to test whether the virus is still infectious.

Implementation

It is already known that the DABCO treated cloths have anti-bacterial and anti-fungal abilities. The optimal length of the attached chain differs between bacteria and fungus. In addition, it seems that the diDABCO-C4 chain is the most effective at inactivating viruses. The molecules can be attached to several different surfaces and multiple molecules can be attached to the same surface; this can create a unique antimicrobial surface that can be used in many different settings. One such setting is hospitals where both fabrics and surfaces could be treated with DABCO and lower the amount of microorganism spread by contact. Furthermore, air filters could be treated to inactivate or kill microorganisms before they spread around a hospital. However, before this can be implemented the antiviral mode of action need to be understood. This is important because once the fabric or air filters are used, they must be discarded in a safe

manner. If the virus is permanently inactivated the air filters might be used more than once, whereas if the virus is only temporarily inactivated the air filters would only be used once and then be discarded as biohazards wastes. These types of air filters might be useful on airplanes where individuals spend hours sitting in circulated air. If a DABCO treated filter were in place, it might be able to bind the microorganisms and prevent them from circulating and infecting passengers. Lastly, the military might be able to use this type of treatment on their uniforms if in areas where biological weapons or deadly diseases are of high risk. Additionally, using DABCO treated gauze could lower the chance of infection for soldiers injured while on patrol.

Someday the DABCO-conjugated molecule may be used in medical treatments. Unfortunately, there is no truly effective treatment or medication for viral infections at the moment. DABCO-conjugated molecules might be able to be used as a treatment, in the future. If we can find a way to line a blood bag or design a filter to “clean” infected blood, the DABCO-conjugated molecule might be able to change how we treat viral infections. It could also be used for donor blood to ensure the blood does not contain any viruses before being bagged, stored and used for blood transfusions.

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Figures

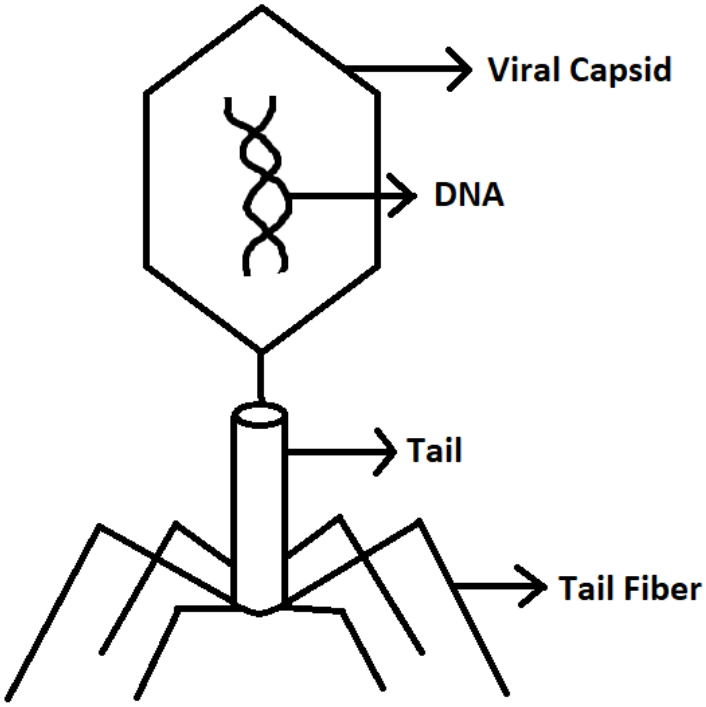

Figure 1. Structure of Bacteriophage T4. The virus consists of a protein capsid that encloses the viral DNA. The capsid is attached to the tail which have protein fibers that are used for recognition, anchoring, and infection of *E. coli*.

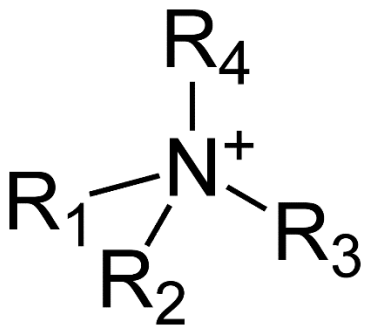

Figure 2. Molecular structure of a quaternary ammonium compound (QUAT). QUATs are positively charged because the Nitrogen atom is attached to 4 hydrocarbon chains. These hydrocarbon chains can be specifically modified to obtain antimicrobial effects.

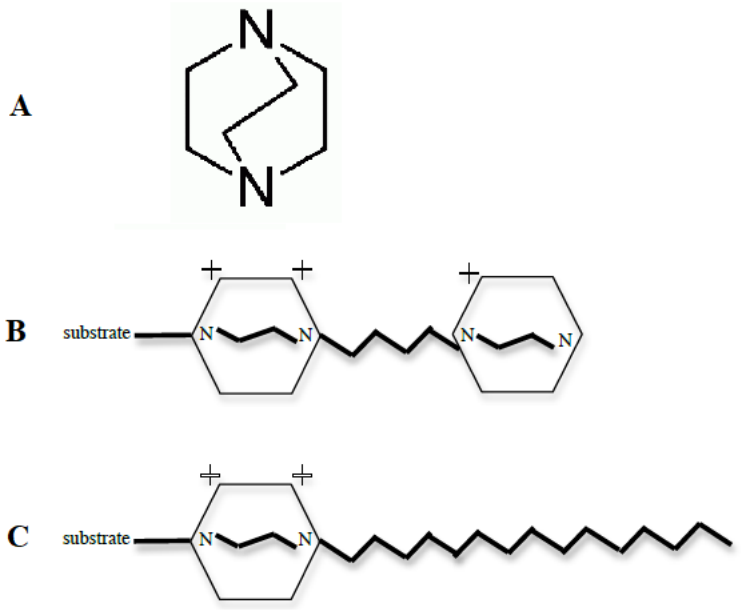

Figure 3. Different types of DABCO-conjugated molecules. (**A**) The basic structure of a DABCO molecule. (**B**) diDABCO-C4 molecule consisting of two DABCO molecules connected by a hydrocarbon chain of four carbons. (**C**) DABCO-C16 molecule consisting of a DABCO molecule with a hydrocarbon chain of 16 carbons.

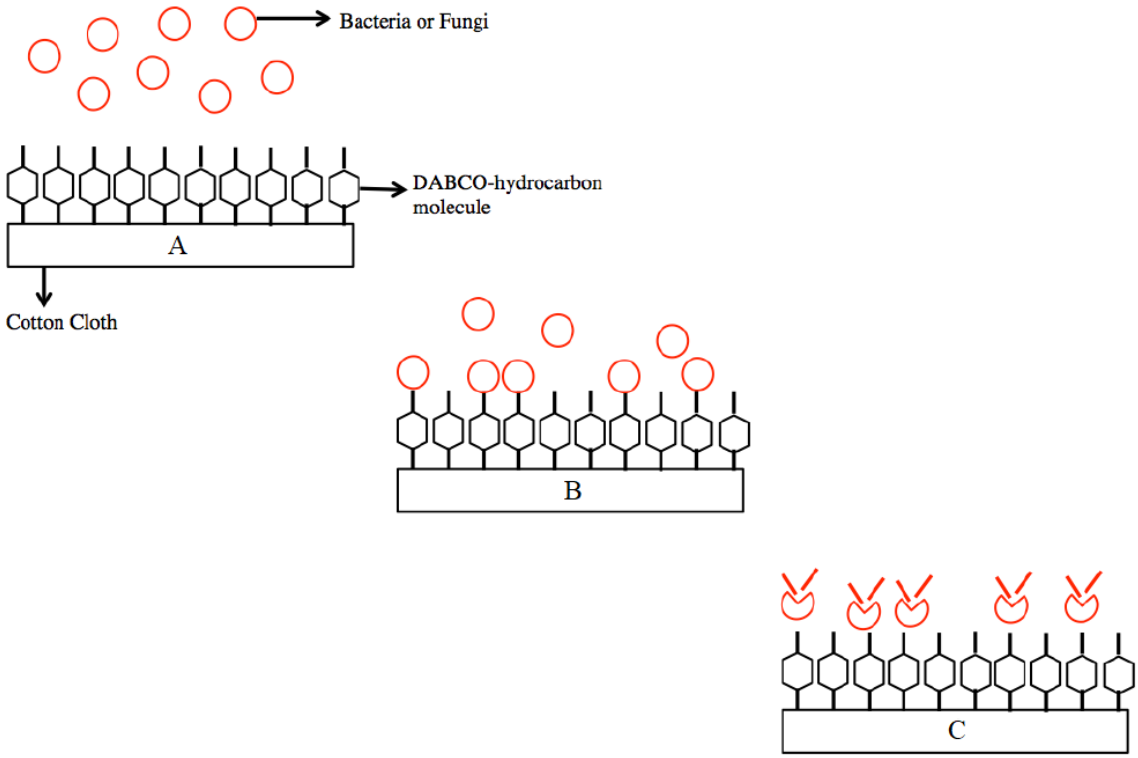

Figure 4. Mode of action by DABCO-conjugated molecules on bacteria or fungi. (**A**) Bacteria or fungi with negatively charged cell walls are attracted to the DABCO-conjugated molecule. (**B**) The bacteria or fungi move closer and contact the DABCO-conjugated molecule. (**C**) The DABCO-conjugated molecule pierces the cell wall and the bacteria or fungi lyse.

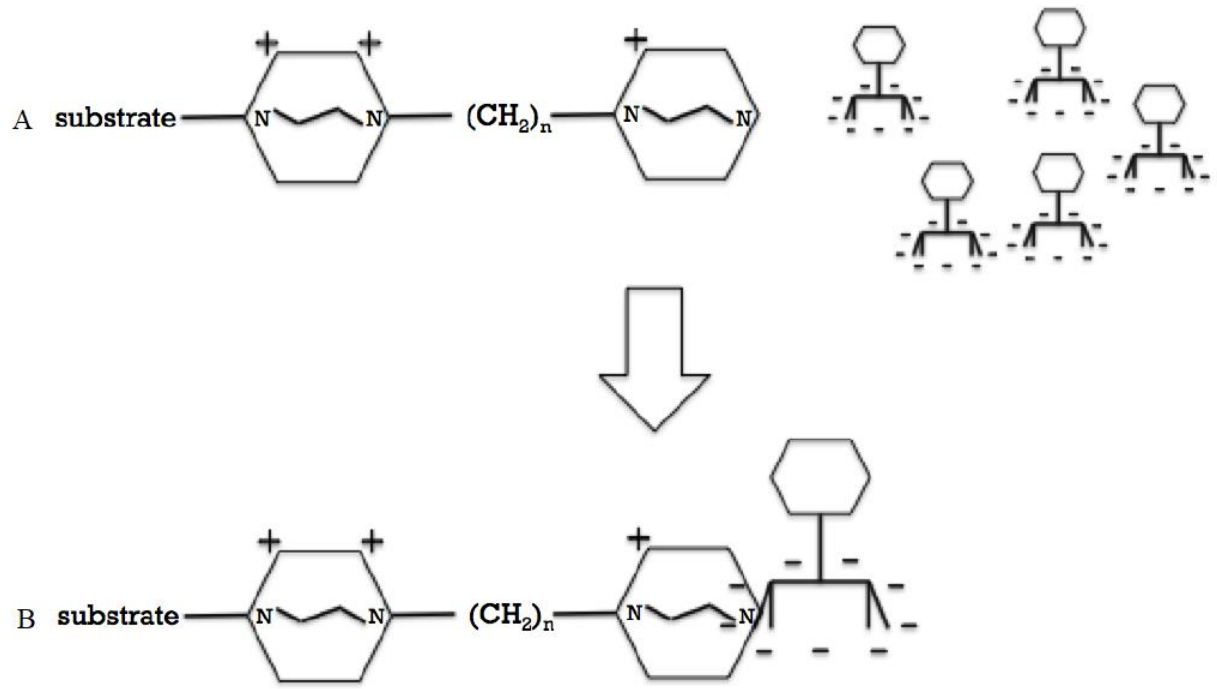

Figure 5. Proposed mode of action of DABCO-conjugated molecules on T4 bacteriophages. (**A**) The diDABCO-conjugated molecule carries a strong positive charge, which attracts the negatively charged T4 bacteriophages. (**B**) The T4 bacteriophage interacts with the molecule through an electrostatic interaction, which ultimately removes it from the environment.

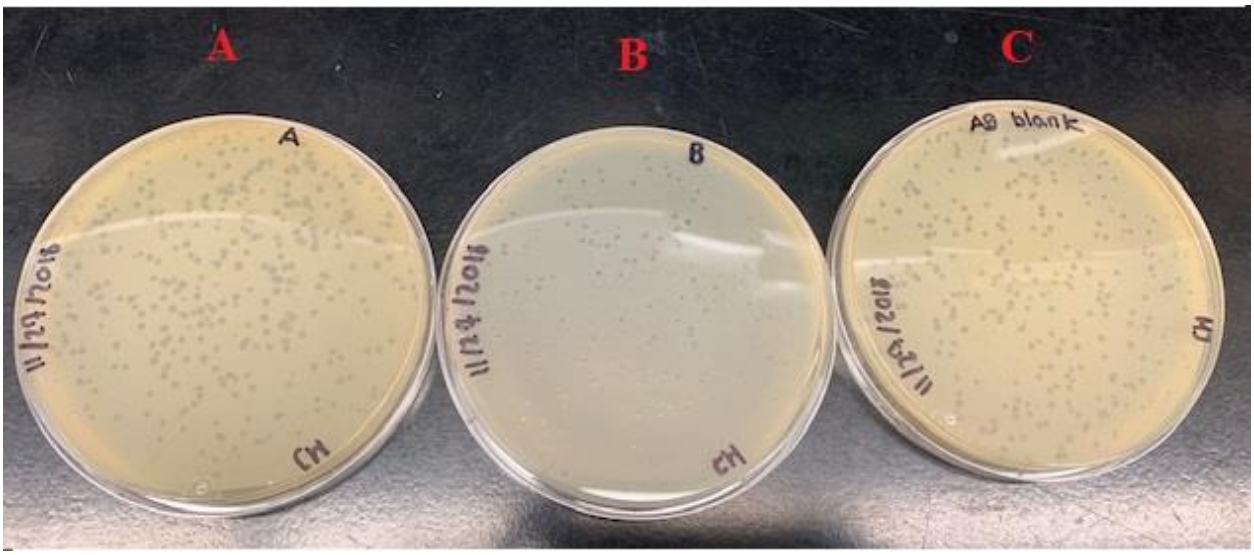

Figure 6. Partial results from the evaluation of DABCO cloths. (**A**) 322 plaques present after treatment with diDABCO-C4 cloth A. No reduction in viral load seen. (**B**) 152 plaques present after treatment with diDABCO-C4 cloth B. 46% reduction in comparison with the untreated cloth. (**C**) 284 plaques present after control treatment with blank/untreated cloth.

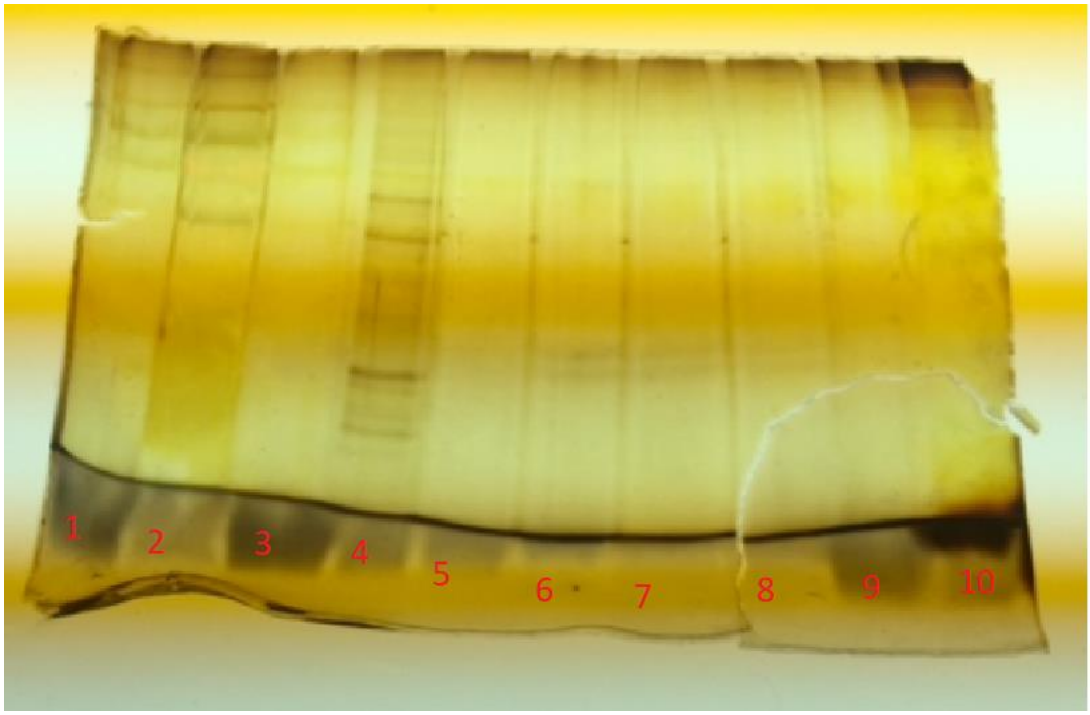

Figure 7. Silver stained gel electrophoresis results. Gel electrophoresis results from previos research (Stirling, 2017). Lane two and ten contained the ladders used for comparison. Lane one, three, and nine were left empty. Lane four was loaded with a 10^{-1} viral dilution. Bands in this lane was used as a positive control. Lane five was loaded with a solution resulting from treatment with a blank cloth, but no detergent and virus. This was used as the negative control. Lane six contained a solution resulting from treatment with diDABCO-C4, but no detergent and virus; another negative control. Lane seven was loaded with the sample resulting from blank cloth with virus and SDS treatment. Lastly, lane eight was loaded with the diDABCO-C4 and virus with SDS treatment.