In vitro assessment of the antiviral and antibacterial potential of Silver (Ag), Titanium (Ti), and Copper (Cu) based nanoparticles for the development of bio-active fabrics.

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In vitro assessment of the antiviral and antibacterial potential of Silver (Ag), Titanium (Ti), and Copper (Cu) based nanoparticles for the development of bio-active fabrics.

A thesis submitted in partial fulfillment of the requirement for the degree of Master of Sciences in Healthcare Biotechnology

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DECLARATION

I, Daniyal Sajid Khan, certify that the research work presented in this thesis titled *"In vitro assessment of the antiviral and antibacterial potential of Silver(Ag), Titanium(Ti), and Copper(Cu) based nanoparticles for the development of bio-active fabric."* is the result of my work. Information has been derived from other sources; it has been properly acknowledged/mentioned in the thesis. The work herein was carried out while I was a post-graduate student at Atta-ur-Rahman School of Applied Biosciences, NUST.

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DEDICATED TO

My beloved family

For their support throughout the journey

All my Teachers to date

For their role in making me who I am today,

especially,

Dr. Sobia Manzoor

&

Dr. Madiha Khalid

The two women who mean the most to me

Forever thankful for their support & guidance

&

Me, Myself & I

For the perseverance,

compassion, and the resolution to

never give up

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"My success is only by ALLAH"

Qur'an [11:88]

~Daniyal Sajid Khan

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List of Acronyms

1. **AgNP** (Silver Nano-particles)
2. **ATCC** (American Type Culture Collection)
3. **CFU** (Colony Forming Unit)
4. **DNA** (Deoxyribonucleic Acid)
5. **DMEM** (Dulbecco's Modified Eagle Medium)
6. **FBS** (Fetal Bovine Serum)
7. **ISO** (International Organization for Standardization)
8. **MH** (Mueller-Hinton)
9. **PPE** (Personal Protective Equipment)
10. **PFU** (Plaque Forming Unit)
11. **PBS** (Phosphate Buffer Saline)
12. **PCR** (Polymerase Chain Reaction)
13. **PHE** (Phenylalanine)
14. **ROS** (Reactive Oxygen Specie)
15. **RNA** (Ribonucleic Acid)
16. **SARS** (Severe Acute Respiratory Syndrome)
17. **TCID50** (Median Tissue Culture Infectious Dose)
18. **UTM** (Universal Transfer Media)

ABSTRACT

The frequent occurrence of various microbial diseases poses a threat to the modern world. Microbial pathogens consist of a wide variety of bacteria & viruses. These pathogens exist throughout the globe and affect the health of millions annually. Frontline workers, who are in first-hand contact with these microbes, work hard to control the disease condition by playing their role in disease prevention and treatment. This puts these front-line workers at more risk against these viral and bacterial strains. Air-borne microbes are easily transmitted and are more difficult to control. Most of the work has been done on disease prevention worldwide and the treatment mainly includes antibiotics to ensure proper safety against these fatal and harmful microbes, personal protective equipment (PPEs) are specially designed for these workers to kill or inactivate these microbes. The study focuses on the testing of the anti-bacterial potential of e-textiles coated with copper and titanium-based nano-particles and the anti-viral potential of e-textiles coated with silver nanoparticles. The in-vitro assessment of bioactive fabric, comprised of different nanoparticle coatings, was done against Gram-positive (S. aureus ATCC 6538), Gram-negative bacteria (E. coli ATCC 8739), and Corona virus. Standard bacterial agents were tested with ISO 20743 norm and ISO 18184 was used for anti-viral testing. A sample size of n=9 was assessed based on qualitative and quantitative analysis. The qualitative analysis was based on the measurement of zone of inhibition in comparison to gentamycin standard. The qualitative results were measured as reduction in the CFU number of the bacteria in terms of decimal logarithm average per milliliter assessed at different time intervals. The anti-viral results were analyzed using the Spearman-Karber equation and the average reduction between the log infectivity titer and log reduction titer was calculated which was presented in terms of percentage reduction. Most copper samples showed excellent results with an average of 20mm zone of inhibition and more than 90% reduction in CFU number. Titanium samples

showed ordinary results apart from TOSAC, showing comparable results to copper samples. The anti-viral load was also reduced by more than 80% in most cases using the silver coated nano-fabrics. The experiments were run in triplicates and the samples were subjected to testing both prior and after washing so the integrity of the fabric is also checked. The study provided intuition about anti-microbial activity of different e-textiles for their use in Personal Protective Equipment (PPE)

Keywords : Nano-particles ; Anti-bacterial ; Anti-viral ; TCID-50 ; PPE ; COVID-19 ; *S.aureus* ; *E.coli* ; ISO 18184 ; ISO 20743

Chapter1

INTRODUCTION

Covid-19 is an enveloped virus of the family Coronaviridae, which causes respiratory tract infection in humans along with cough, cold, sneezing, and in some instances, diarrhea (Kumar, Malviya, & Sharma, 2020). Coronavirus has a single-stranded RNA genome inside a spherical envelope with club-shaped glycoprotein structures to aid in attachment. Found in four sub-types, i.e., alpha, beta, gamma, and delta, the virus has infected around 240 million people worldwide. Various vaccines have been offered for COVID since the pandemic and several drugs. As the virus targets the lungs, oxygen ventilators have been used in severe cases. The virus has an airborne transmission that is difficult to control, and the prevention measures include masks, frequent sanitization, and personal protective equipment (Daniel, 2020).

Nanoparticles have become prime importance for researchers of different fields (Narayanan & Sakthivel, 2010). “Nano” is derived from the Greek word “Nanos,” referring for dwarf , and stands for a measurement of one-billionth a meter (Thakkar, Mhatre, & Parikh, 2010). A single strand of deoxyribonucleic acid is 2.5 nm in diameter (Wagner, Plank, Zatloukal, Cotten, & Birnstiel, 1992). A typical virus is around 100 nm wide, whilst a bacterium is approximately 1-3 µm in width (Katz et al., 2003). Nano-particles are dispersions of solid matter in particulate form with having at least a single dimension with in the size range of 10-1000 nm (Mohanraj & Chen, 2006). The most crucial feature of nanoparticles is their comparative surface area to the volume ratio, allowing them to easily interact with other solid particles. Organisms have opted several mechanisms of evolution to cope with environments containing high levels of metals (Pantidos & Horsfall, 2014). Diagnosis, treatment, and drug delivery have been made trouble-free using nanoparticles. The similar size range of nanoparticles and various biomolecules, such as nucleic acids and proteins, allows nanoparticles to function more efficiently at a molecular level. Bio-sensing, extra and intracellular process inhibition, and targeted drug delivery have been a few of the significant roles of nanoparticles along with bacterial and viral inhibition(De, Ghosh, & Rotello, 2008).

Antibacterial, viral, and fungal activity of nanoparticles has been previously established in many studies such as silver, gold, and silica (Draz & Shafiee, 2018; Greulich et al., 2012; Paulo, Vidal, & Ferreira, 2010). Nanoparticles can have one or both bio-static or biocidal effects as there is a decline in the number of effective antibiotics available and an elevation in resistance against antibiotics. Hence, the focus of researchers is on nanoparticles being potential new medicinal gears. Nanoparticles of silver have also been used as optical sensors to form small molecule adsorbates (McFarland & Van Duyne, 2003). In contrast, platinum based catalysts have shown high activity when electro-oxidation of formic acid was carried out. (Waszczuk, Barnard, Rice, Masel, & Wieckowski, 2002).

The COVID-19 pandemic required workers' active contributions to prevent this situation from spreading exponentially as health workers are in constant contact with the virus, so the viral testing was done on a massive scale daily. Such problems are menacing for the patients and put the health workers in a dangerous position(Daniel, 2020). Personal Protective Equipment with antibacterial and antiviral activities are a helpful advancement in the field of PPE's as they enhance the protective ability of this equipment. Fabrics coated with nanoparticles have been proved more effective than traditional fabrics for PPE use (Basodan, Park, & Chung, 2021). Metal nanoparticles are immobilized on the materials via different methods made into Personal Equipment (Cioffi et al., 2005).

Copper nanoparticles have been used widely for their non-toxic nature, easy synthesis, and cheap cost with effective antimicrobial activity.(Rafique et al., 2017). Titanium nanoparticles, especially titanium dioxide nanoparticles, are produced on a large scale worldwide. Titanium nanoparticles are most effective under visible light as they require low excitation energy to acquire an active state under which they interact best with the biomolecules. Titanium nanoparticles have become an essential component in water-purification filters.(Lan, Lu, & Ren, 2013). Silver has been recognized primordially for its antibacterial activities. Applying

silver in a nanotechnology-based structure helps its effective range and enhances its variety of applications (Beyene, Werkneh, Bezabh, & Ambaye, 2017). This study aimed to test nanoparticle-coated fabrics against infectious bacteria and viruses to develop personal protective equipment.

With different bacterial and viral microbes present, health workers can get infected by these threatening biological agents. Proper safety equipment is necessary for such field workers to have appropriate protection against these infectious agents. Personal Protective Equipment (Lippi & Plebani) includes gloves, goggles, facemasks, and a full-body protective suit (Organization, 2020b).

Titanium, silver, and copper nanoparticles coated fabrics will be tested in this study. Here, the antibacterial activities of e-textiles are under testing. Both gram-positive and gram-negative bacteria will be tested after particular time intervals. For gram-positive bacteria, S. aureus (ATCC 6538) will be used, and for gram-negative bacteria, E. coli (ATCC 8739) will be used. It is well known to cause several diseases like cellulitis, diarrhea, respiratory illness, and skin infections; the bacteria mentioned above are used for standardized testing. The mechanisms by which metal nanoparticles cease bacterial growth include releasing reactive oxygen species and cations, which causes cell apoptosis, ATP depletion within mitochondria, and plasma membrane degradation. The percentage reduction in the bacteria's Colony Forming Units (CFU's) will be measured in decimal logarithm average per milliliter at different time intervals, i.e., instantly after contact with the fabric (0 Hour) and after 24 hours of incubation with the material. The antiviral activity will be tested against the notorious COVID-19 viral particles. Viral load will be maintained via TCID50 is incubated in contact with the fabric, and the viral load reduced will be measured.

This study to develop bioactive e-textiles that would prove to be an excellent preventive measure against infectious biological agents, reducing the need for antiviral and antibacterial drugs and helping the public and health workers avoid contagious diseases.

Chapter2

Literature Review

2.1 COVID-19

SARS CoV-2 is the seventh human coronavirus. The virus was Discovered in Wuhan, Hubei province of China in January 2020 (Wu et al., 2020; Zhou et al., 2020). The virus has been categorized as a pandemic since then; by 20th May 2020, 4.8M people got infected and caused 0.31M deaths(Fahmi, 2019). COVID-19 , SARS-CoV and MERS-CoV cause severe pneumonia with a mortality percentage of 3%, 9.6 percent, and ∼36 percent, respectively(Azhar, Hui, Memish, Drosten, & Zumla, 2019; David SC Hui & Zumla, 2019; C. Wang, Horby, Hayden, & Gao, 2020). The rest of the coronaviruses, OC43, NL63, HKU1, and 229E, generally cause self-restricted illness with mild symptoms (Corman, Muth, Niemeyer, & Drosten, 2018). Comparing its genome with other family members provides information about its unique features (Andersen, Rambaut, Lipkin, Holmes, & Garry, 2020). The ribonucleic acid sequence targets the "acid-binding domain" of the "spike protein" (S), suggesting that it arose from a "natural evolutionary process". (Nao et al., 2017).

2.2 Symptoms of COVID-19

People suffering from COVID-19 can have symptoms from moderate to intensive, largely being asymptomatic carriers. Common reported symptoms include fever and cough (83%) with shortness of breath in 31% population. (D. Wang et al., 2020). A chest X-ray usually shows molting at multiple sites and a opacity of ground-glass level. (Zhu et al., 2020). 2-10% of the patients also showed symptoms of the Gastro-intestinal level including diarrhea , vomiting and abdominal site pain .The GI track symptoms lead to development of fever and respiratory symptoms in around 10% pateints. (D. Wang et al., 2020).

2.3 Diagnosis of COVID-19

A decreased level of lymphocyte and eosinophils was observed in COVID-19 patients. Levels of CRP, ALT ,LDH and AST were observed to be lowered along with CRP levels in the serum.(Lippi & Plebani, 2020). Initial CRP serum levels have proved to be and be a sole predictor for the diagnosis of severe COVID-19 infection. (Bhargava et al., 2020; C. Z. Wang, Hu, Wang, Li, & Li, 2020). The main target of the coronavirus infection is the lung, being the source of large number of ACE-2 receptors. (Hamming et al., 2004) This may lead to heart, stomach, liver, kidney, brain, and eye damage that has to be monitored. (Renu, Prasanna, & Gopalakrishnan, 2020). Observing the cardiac-troponin levels may be helpful. Patients with the "acute respiratory distress syndrome" can lead to "multiple organ failures" induced by the so-called "cytokine storm" (D. Wang et al., 2020).

2.4 Treatment of COVID-19

The cytokine data resembling the "secondary hemophagocytic lymphohistiocytosis syndrome" has been found similar to severe COVID-19 cases. It is characterized by increased IL-2,7 "granulocyte colony-stimulating factor" , "interferon-γ inducible protein-10" , "monocyte chemoattractant protein 1" , "macrophage inflammatory protein 1-α" , and "TNF-α" (Huang et al., 2020). Death is likely to occur due to hyperinflammation induced by the virus which is predicted by increased rate of IL-6 and ferritin levels. (Ruan, Yang, Wang, Jiang, & Song, 2020). Relying on it, tocilizumab (IL-6 receptor blockade) was given to a person suffering from COVID-19 with increased serum IL-6 to decrease lungs inflammation.(Ciotti et al., 2020).

The "Italian Agency of Medicine (AIFA)" recently approved a clinical trial .To prevent thromboembolism-related complications , Enoxaparin is given subcutaneously to patients with COVID-19 (Lang et al., 2011). Heparin is also known for its ability to contain a viral infection, including coronavirus. In the company of heparin, the S protein interaction with heparan sulfate

may be blocked, resulting in no cellular entry. Moreover, heparin inhibits proteases which have a role in virus infectivity. (Belen-Apak & Sarialioglu, 2020).

This disease in children is generally asymptomatic or mild compared to that seen in adults for reasons yet to be illuminated. The clinical laboratory data of children is way more diverse than adults. An inconsistent alteration of the leukocyte index was recently reported in a meta-analysis (Henry et al., 2020); though, increased CRP, procalcitonin, and LDH levels have also been determined in kids. Remarkably, creatine kinase-MB was elevated in one-third of patients, raising the suspicion of cardiac involvement in COVID-19 pediatric patients, as recently reported (Sanna et al., 2020).

2.5 Prevalence of COVID-19 across the globe

Dreadfully, many of the infections and deaths recorded in this global outbreak have occurred in healthcare workers. HCWs account for 9% and 13% of the total confirmed COVID-19 cases in Italy and Spain, respectively (Chustecka, 2020; Fasogbon et al.; Giuffrida & Tondo, 2020). On 17th April 2020, the Italian National Institute of Health announced that nearly 17,000 HCWs in Italy had contracted the illness (Thomas et al., 2020). Shortly afterward, the Italian Federation of Medical Associations (FNOMCeO) reported that 139 Italian doctors died of the disease(Bramstedt, 2020). As of 28th April 2020, the Government has verified 49 deaths among NHS HCWs due to COVID-19 during the pandemic in the UK. However, other organizations report that the figure may have surpassed 100 deaths(Marsh, 2020).

2.6 Spread of SARS-COV-2

Like other respiratory viruses, SARS-CoV-2 transmission occurs with high efficacy and infectivity, mainly through the respiratory route (Leung et al., 2020). Severe acute respiratory distress syndrome virus's ribonucleic acid was present on non-living shells like door handles,

cell phone surfaces in residential sites of patients with confirmed COVID-19. Although aerosols may represent another critical route. (Han, Lin, Ni, & You, 2020).

The vertical mode of COVID-19 transmission is still under discussion. No mother-to-child transfer was observed in a set of nine COVID-19 positive pregnant women. No viral partciles were observed were in the breast milk indicating that it is not a carrier of the virus from mother to child. (H. Chen et al., 2020). However, a newborn with high levels of IgM against COVID-19 born to a positive mother was reported , when tested two hours after birth. Increased Interleukin-6 and Interleukin-10 were observed. When polymerase chain reaction (PCR) was executed on repeated nasopharyngeal wipes from two hours to sixteen days of age, negative results were observed. The hypothesis was proposed that "IgM cannot cross the placenta and be transferred to the fetus", which indicates that the newborn was infected *in utero.*(Dong et al., 2020).

Another virus transfer route of the COVID-19 virus could be the eyes. Ribonucleic acid of the respective virus was detected in ocular swabs at time period of 3 days and 27 days after infection. Interestingly, the UTM from an ocular swab from a positive patient was propagated in "Vero E6" cells, suggesting that ocular secretions are infectious. (Colavita et al., 2020). The research suggesting that eye protection should be sued when examining COVID-19 patients.(Lu, Liu, & Jia, 2020).

2.7 Life cycle of COVID-19

As shown in ***Fig.2*** , Coronavirus has an envelope, capsid, and other proteins including E protein, S protein, N protein, M protein and HE protein that are mainly involved in their entry and mechanism of interaction with the host cell. The virus gets entry into the host lungs through the eyes or nose. Alveoli of lungs containing ACE-2 Receptor with which spike proteins of virus make interaction. Surfactants produced by type 2 pneumocytes cause a reduction in

pressure and surface tension in alveoli. Host cell proteases cleave the spike proteins of viruses and assist in their entry through two mechanisms either endocytosis or membrane fusion. After their entry, positive-sense RNA is released in the cytoplasm of the host cell where it is being

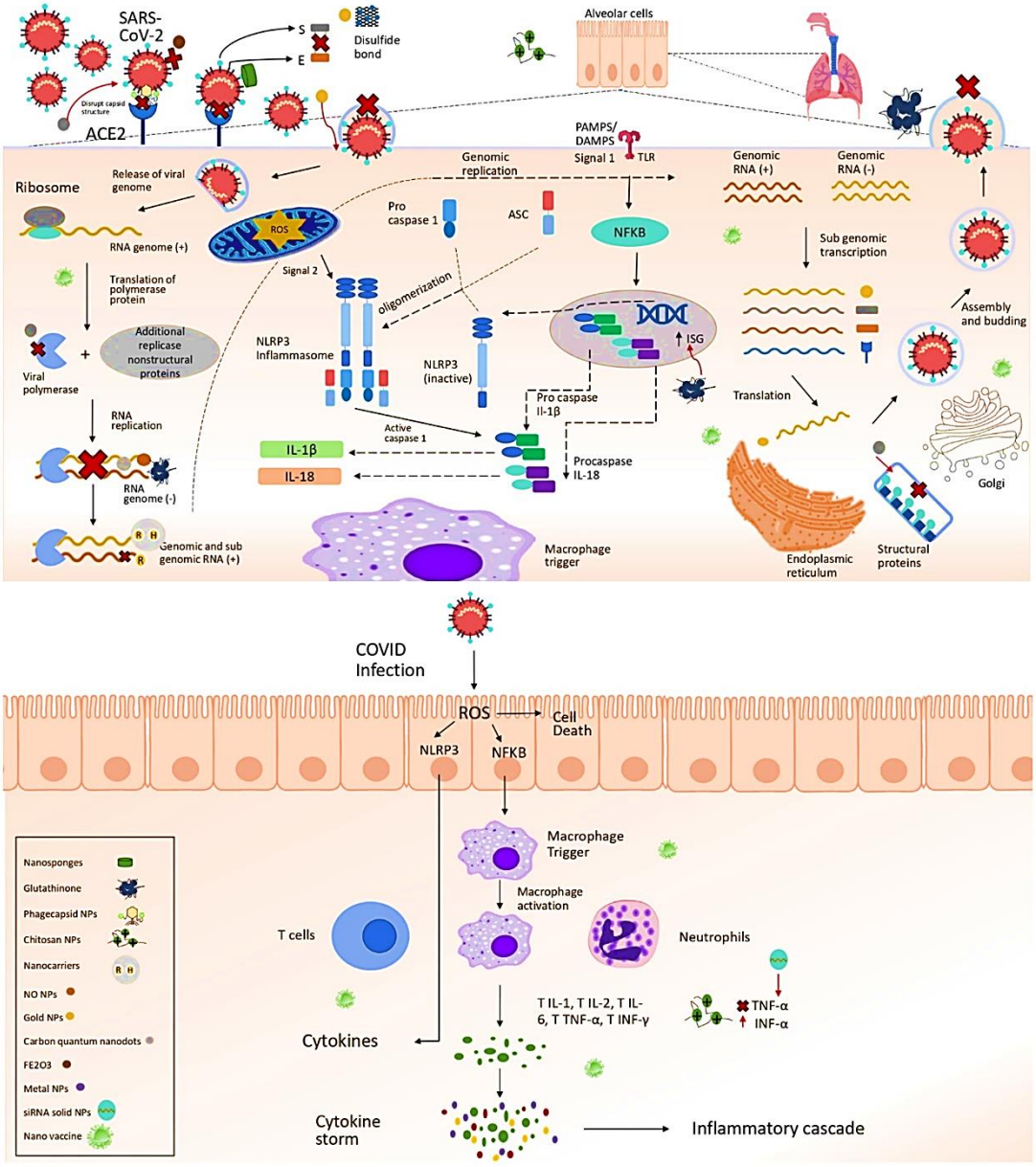


Fig. 2. Life Cycle of SARS-COVID-19

replicated to –RNA in the presence of RNA-dependent RNA polymerase, along with a translation of polyproteins pp1a and pp1ab. These polyproteins help in the replication and transcription of viral RNA. Negative sense RNA is either incorporated in the viral genome, direct conversion to positive-sense RNA (+RNA) or translated to viral proteins including spike

membrane, envelope and nucleocapsid proteins. These viral proteins are sent to the Golgi apparatus for packaging through the endoplasmic reticulum of the host cell. Eventually, being assembled near the host cell membrane. Complete virion exit from host cell through exocytosis, resulting in the death of host cell (Boopathi, Poma, & Kolandaivel, 2021). Metal nanoparticles (iron oxide, zinc oxide, carbon-based, copper nanoparticles) have shown antiviral properties by interfering with viral attachment and binding to host cell, inhibit the viral replication process via disruption of virus capsid and halted the genome synthesis process.

2.8 Preventive measures against COVID-19

Frontline organizations such as the "British Medical Association (BMA)" and "Doctors' Association UK (DAUK)" have consistently voiced their fears. According to them, the national personal protective equipment (PPE) guidelines issued by Public Health England (Davidson et al.) (Holland, Zaloga, & Friderici, 2020) are inadequate to deliver supreme defense in quantitative and qualitative ways. Despite many changes to the personal protective equipment supervised by PHE from the beginning of the pandemic, immobile alarms show the PPE's efficacy (Thomas et al., 2020).

2.9 The logical basis of PPE

2.9.1 Spread

Current evidence indicates that SARS-CoV-2, as with SARS-CoV-1, may be spread by four notable routes: direct or fomite contact; respiratory droplets generated during coughing or sneezing; fecal-oral; and airborne via aerosols (Ong et al., 2020; Organization, 2020a). Airborne and fecal-oral transmission can result in super-spreading events – particularly in

nosocomial settings (Chan, Yuan, & Kok, 2020; Tellier, Li, Cowling, & Tang, 2019; W. Wang et al., 2020; Yuen, Ye, Fung, Chan, & Jin, 2020).

2.9.2 Aerosols

Recent studies report activities including oxygen mask adjustment, physical examination, and intravenous access are similarly aerosol-generating (Weber, Phan, Fritzen-Pedicini, & Jones, 2019). A more recent study showed that viral RNA could be found in respiratory droplets and aerosols generated during exhaled breaths from influenza, coronavirus, and rhinovirus-infected patients (Leung et al., 2020). Another study found that influenza RNA concentration surpassed the infectious dose in all sampled clinical areas providing routine care in a tertiary hospital(Bischoff, Swett, Leng, & Peters, 2013). HCWs were exposed to mainly small influenza virus particles (diameter <4.7 μm even at distances of 1.829 m from patients (Bischoff et al., 2013).

SARS-CoV-2 has been shown to remain viable as bio-aerosols for at least 3 hours and on other surfaces (such as cardboard, plastic, and metals) for longer (Van Doremalen et al., 2020). Thus with current evidence yet to conclusively elucidate the nature and degree of airborne spread of SARS-CoV-2,32 PPE guidance that risk-stratifies based on the aerosol-generating potential of clinical activity at specific clinical locations or proximity to a patient may be vastly underestimating the risk. For instance, even in 'low' or 'intermediate-risk clinical settings, such as general medical wards, HCWs may receive significant viral exposure as they encounter multiple COVID-19 patients shedding unpredictable, viral loads generated through breathing, speaking, or coughing.

2.9.3 Respiratory protection

The major mask manufacturer 3M states that surgical facemasks are inadequate to protect the wearer from inhaling particles smaller than 100 μm, including aerosols and most droplet

particles generated through sneezing or coughing.33 Instead, 3M recommends at least an N95 respirator mask to protect against inhaling particles of this size (Smith et al., 2016).

2.9.4 Body, hand, and eye protection

A systematic review of PPE measures for SARS-CoV-138 reported favorable evidence for eye protection (OR 0.10), gloves (OR 0.32), gowns (OR 0.33), and handwashing (OR 0.54). Combining these methods with respiratory protection was even more effective (OR 0.09) (Jefferson et al., 2020).

In one study, 62% of COVID-19 cases had a history of hand-eye contact, and 4.68% presented conjunctival congestion (L. Chen et al., 2020). While eye protection has primarily been shown to be effective in the context of the respiratory syncytial virus, it has typically been studied when used alongside other PPE (French et al., 2016). Face-shields can potentially simultaneously offer eye and respiratory protection (Roberge, 2016) but provide insufficient protection against aerosols and droplet contamination if used on their own (Lindsley, Noti, Blachere, Szalajda, & Beezhold, 2014).

2.9.5 Cost-effectiveness

Cost-effectiveness modeling has revealed that utilizing enhanced PPE (respirators, gowns, and goggles) for all patients would be cost-effective in the setting of a pandemic (Chandini Raina MacIntyre et al., 2017) such as severe acute respiratory syndrome (SARS) (Dan et al., 2009) by analyzing its importance. But as real-world studies on this subject are limited (C Raina MacIntyre & Chughtai, 2015), data from this ongoing crisis should be evaluated to inform future policies on preparation and stockpiling.

2.10 Nanoparticles and their antibacterial properties

It is well known that inorganic nanoparticles can act as antibacterial and antifungal agents and thus can interact with microorganisms (W. Wang et al., 2020). However, before using microorganisms as a means of transport for bioactive molecules, such as nanoparticles or drugs linked to nanoparticles, it is essential to investigate how to deposit the molecules within microorganisms(Anfinrud, Stadnytskyi, Bax, & Bax, 2020; Nicas, Nazaroff, & Hubbard, 2005; Yuen et al., 2020). It has already been described how Gram-negative bacteria can be used for constructing bacterial ghosts, representing novel advanced delivery and targeting vehicles suitable for the delivery of hydrophobic or water-soluble drugs (Gawn, Clayton, Makison, & Crook, 2008). Also, in pathogenic fungi, like C. Albicans, several components associated with the cell wall have been identified to play an essential role in fungal–host interactions (Bourouiba, 2020).

We have previously demonstrated that a self-assembly process takes place by simple mixing of nanoparticles of gold and platinum with Salmonella enteritidis and Listeria monocytogenes, but with different morphologic interactions depending on the bacteria and nanoparticles used (Tran, Cimon, Severn, Pessoa-Silva, & Conly, 2012). In the present investigation, other microorganisms and additional nanoparticles were chosen to elucidate further the possibilities for constructing microorganism–nanoparticle vehicles(Nicas et al., 2005). Exerting their antibacterial properties, silver nanoparticles (nano-Ag) attach and anchor to the cell's surface (Organization, 2020b; Weber et al., 2019). This interaction causes structural changes and damage, markedly disturbing vital cell functions, such as permeability, causing pits and gaps, depressing the activity of respiratory chain enzymes, and finally leading to cell death (Yan et al., 2018). Nano-Ag inhibits yeast growth [84] and has antifungal activity against different Candida species (Santarpia et al.). Results from toxicologic assays have shown no in vitro cytotoxicity of nano-Ag nanoparticles in concentrations sufficient to inhibit microbial growth

(Lewis, 2020). Still, at high concentrations, nano-Ag do exert cytotoxic effects on human mesenchymal stem cells (Davidson et al., 2013). More and more results provide evidence for a molecular mechanism of nano-Ag activity, showing that nano-Ag acts through reactive oxygen species generation, leading to activation of proteins and inducing apoptosis via the mitochondrial pathway (Lindsley et al., 2012). In vivo measurements in chicken embryos and quails demonstrated that hydrocolloids of nano-Ag at the level of 50 ppm did not affect the development or cause oxidative DNA damage to embryos35 or growth of quails (David S Hui et al., 2012).

2.11 E-Textiles

Textile-integrated conductive materials or electronics, also referred to as intelligent textiles or e-textiles, are a rapidly growing class of products. These hybrid e-textiles can be characterized as textile products with additional functionality provided by electronic or electrically conductive components (Rotzler, Krshiwoblozki, & Schneider-Ramelow, 2021). These added functions are sensors, lighting, heating, stimulation, or recording of internal or external parameters. Simultaneously, e-textiles constitute electronic products with additional—textile typical—requirements, resulting from integration into a textile base(Rotzler et al., 2020). It is especially true for wearable e-textiles, designed to be worn on or close to the body. To ensure wearer well-being, they need to be sufficiently breathable and comfortable. Depending on the application—stretchable enough to withstand a range of mechanical stresses during use—mainly tensile, but also bending, shear, torsion, or compression stress (Reumann, 2000). Like textiles without integrated systems, wearable e-textiles can become stained during their use and therefore need to be cleanable or washable (Bisenius, 2017). During the washing process, characterized by the four interdependent factors of time/duration, mechanical action, temperature, and chemistry/biology, also labeled Sinner's factors (Wehr, 2017), different strain scenarios occur that the e-textile needs to be able to withstand. Especially with a large fraction

of e-textile products developed for medical, personal protective equipment (PPE), and sports applications, resulting in hygiene requirements make washability an essential property (Ohnemus & Rasel, 2018). E-textiles should hold up to a (user-oriented type and the number of (household) washing cycles during their life cycles, or, in the case of medical or PPE products, even industrial washing if applicable (Rotzler, 2018).

2.12 E-Textiles in relation to the nanoparticles and PPEs

Personal protective equipment (PPE) offers wearers protection from safety and health hazards. A wide range of occupational groups, including healthcare providers, police/military forces, firefighters, and construction workers, require different types of protection and wearability needs (Basodan et al., 2021). For example, firefighters do not typically encounter the pathogens healthcare workers do, while healthcare workers are not expected to walk through flames as firefighters are (Yetisen et al., 2016). Nanotechnologies have addressed PPE-specific needs that were impossible to resolve previously (Kośla, Olejnik, & Olszewska, 2020). There have been remarkable nanotechnology-enabled breakthroughs in smart textiles (Shi et al., 2020; Tao, 2001)and wearable sensors during the last two decades (Khan, Ostfeld, Lochner, Pierre, & Arias, 2016; Ray et al., 2019). These breakthroughs facilitate the development of intelligent PPE (Tricoli, Nasiri, & De, 2017). Here, intelligent textiles refer to textiles that respond to external stimuli in a manual or pre-programmed manner (either with or without integrated microelectronic systems) (Stoppa & Chiolerio, 2014). Wearable sensors may detect kinematic, physiological, optical, and chemical information in the environment to provide the optimal safety to smart PPE wearers; these sensors may be in a flexible/stretchable electronics format (Gao, Yu, Yeo, & Lim, 2020) or a rigid microelectronics format (Stoppa & Chiolerio, 2014).

Chapter3

METHODOLOGY

3.1 Methodology of the study

This study was performed to assess the antibacterial and antiviral potential of nanoparticle-coated e-textiles. The research was conducted in (Atta-Ur-Rahman School of Applied Biosciences) ASAB, NUST in collaboration with National Textile University (NTU), Faisalabad. The antiviral research was performed in KRL Hospital, Islamabad. The approval was taken from all organizations prior performing the study.

3.1.1 Study subject

To evaluate efficacy of fabric samples coated with Titanium, silver and copper-based nanoparticles.

3.1.2 Sampling Procedure

3.1.2.1 Collection of samples:

100 % pure cotton coated with titanium, silver and copper nanoparticles was obtained from National Textile University, Faisalabad. Titanium nanoparticles were immobilized on cotton fabric using pad-dry cure method and silver, copper nanoparticles were contained on the fabric using electroless deposition method.

3.2 Anti-bacterial Testing

3.2.1 Materials

Stock plates of gram negative bacteria (E. coli ATCC 8739) and gram positive bacteria (S.aureus ATCC 6538) , glass Petri plates, falcon tubes, vortex, wire loop, spirit lamp, Pipettes, glass spreader, incubator. Growth media (broth and agar) , Phosphate buffer saline (PBS) were made in distilled water in the lab. All the solutions and glassware were autoclaved for 45 minutes at 121°C at 15psi.

3.2.2 Protocols for bacterial media preparations:

For making broth and agar standard recipes were used. The details are included in Appendices (Appendix-B).

3.2.3 Bacterial enrichment

Mueller-Hinton agar and Mueller-Hinton broth were used for bacterial growth. Petri plates with MH agar were made by pouring 25ml of hot agar into plates one and were refrigerated until needed. The bacteria was streaked in quadrants by loop on media plates and labelled with their respective sample ID. The streaked petri dishes were incubated in an incubator for 18h and temperature was pre-set at 37°C.

3.2.4 Bacterial Isolation

After 18 hours, bacterial colonies were observed on incubated plates. Pure colonies were achieved after repeated quadrant streaking for further usage.

3.2.5 Calculation of colony formation potential

For counting the CFUs, agar over lay method was used. Twenty Serial dilutions of bacterial suspension were formed i.e. 10^{-1} to 10^{-20}. 100µl of each bacterial suspension was added to the respective agar plate along with 20ml of melted soft agar. Continuous clockwise and anti-clockwise rotation of the plate ensured proper mixing and the plate was rested until the agar is set. The plates were then incubated at 37°C for 18 hours. Colonies of all twenty plates were then counted. The bacterial plate with CFUs ranging from 100-300 was used.

3.2.6 One step growth curve

Bacterial log phase was observed for proper experimentation. A primary culture was achieved by adding single bacterial colony to 50ml of MH broth overnight at 37°C. The optical density of the overnight culture was measured at 600nm with sterile MH broth as standard. OD ranging from 0.05 to 0.3 is required according to the protocol by (Y. Gong et al., 1996). The primary culture was diluted accordingly, and the OD was taken every half hour to ensure that the bacteria has acquired a log phase.

3.2.7 Calculation of CFU/ml

The Colony forming unit potential of an overnight culture was measured by the formula:

The number of visible colonies * Dilution Factor / Volume of plated culture in ml= CFU/ ml

3.2.8 Antibiotic standard

Gentamycin was used as the antibiotic standard in the experimentation both against E.coli and S.aurues. Disk diffusion method was used to check the strains against the antibiotic.Both the strains showed clear zone of inhibition when the antibiotic was placed in agar plates with well spread bacterial lawn.

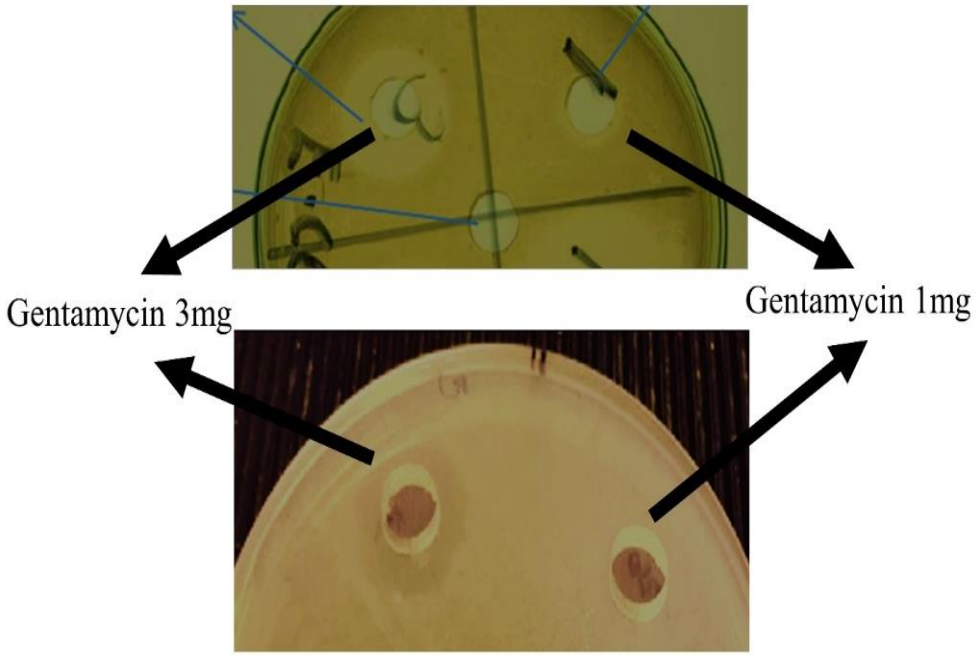


Fig. 3. Sensitivity of bacterial strains was checked against Gentamycin.

3.2.9 Qualitative Assay

The number of bacteria were diluted to achieve the range of 1-3.5x10^6. 100X dilution of the primary culture was made in PBS to get the secondary culture. For this a bacterial colony from the standardized plate with known CFUs was picked and diluted in 50ml PBS. The number of bacteria estimated in the secondary culture were 3x10^8 with an optical density of 0.3. Rigorous vortexing was done to homogenize the solution. 100µl of the secondary culture was pipetted in the center of the pre-set agar plate and a sterile glass spreader was used to evenly spread the inoculum. The sample fabric was then placed on the agar surface and the plates were closed with parafilm and placed in incubator overnight at 37℃.

3.2.10 Quantitative Assay

ISO 20743 was followed for quantitative assay. The bacterial population in logarithmic value of colony forming unit per ml (CFU/ml) was calculated at the 0 hour (time of contact) and after 18hours. Both control and sample fabric were tested side by side to calculate the percent reduction in bacterial colonies by the equation given below.

$$\textit{Percentage mortality} = (\textit{LogTt} - \textit{LogCt}) * 100 / \textit{Log}$$

1ml of secondary culture was placed on pre-set Agar plate and the plate was rested for 5 minutes after which the excess inoculum was removed via pipette. The fabric was then placed on the agar surface of the plate with 200gm weight applied to it. The 0 hour fabric was then tested instantly and the 18h fabric was placed in an incubator overnight. The fabric was next vortexed in 20ml PBS. 10 serial dilutions were then made from that fabric treated PBS ranging from 10- ^{1}to 10^{-10} . 100µl from each serial dilution was then spread on agar plate and the plate was sealed with parafilm and incubated overnight at 37℃.

3.3 Antiviral Testing

The antiviral testing was done against COVID-19 Delta Variant propagated on VERO B4 cells maintained with DMEM..

3.3.1 Materials and Chemicals

Dulbecco's Modified Eagle's Medium (DMEM), Fetal Bovine Serum (FBS), Penicillin-Streptomycin (Pen Strap), Trypan Blue dye, Trypsin, Cell culture flasks, 15ml and 50ml falcons, pipettes, filtered pipette tips, serological pipettes, cell culture petri dishes, 96-well plates.

3.3.2 Test Specimen Preparation

A 100% cotton fabric without fluorescent brighteners or any other material was used as mentioned in ISO 18184. An antimicrobial fabric was prepared by immobilizing different kinds of nanoparticles onto the cotton fabric using a direct chemical method. The precursor controlled the concentration of nanoparticles on fabric to give 146μg of nanoparticle per gram of fabric. Test specimens with a mass of 0.40 g ± 0.05 g were considered and cut into pieces approximately 20 mm by 20 mm apiece

3.3.3 Sample Size

Six Ag coated nano-particles were used for anti-viral testing.

3.3.4 Sterilization of specimens

The sterilization process for fabric included high-pressure steam sterilization. Put specimens in vial containers separately, put all vial containers in a metal wire basket, and cover them with aluminum foil. Put caps of vial containers. Put the basket in the autoclave at 121°C and 15psi to sterilize for 15 min. After sterilization, the foil was removed, all vial containers with the specimens were removed, and put in a safety cabinet. Kept the vials for 60 min there for cooling down. After cooling and drying, put the caps on all vial containers and close them.

3.3.5 Sterilization of apparatus

all the apparatus which comes in contact with the cells, the chemicals, or test specimen was sterilized using UV light and 100% alcohol.

3.3.6 Cell Line

Vero B4 Cells (ATCC CCL-81) were used for anti-viral testing. These are African Green Monkey kidney cell lines. The cell line is made immortalized and are susceptible to infection by SARS-CoV-2.

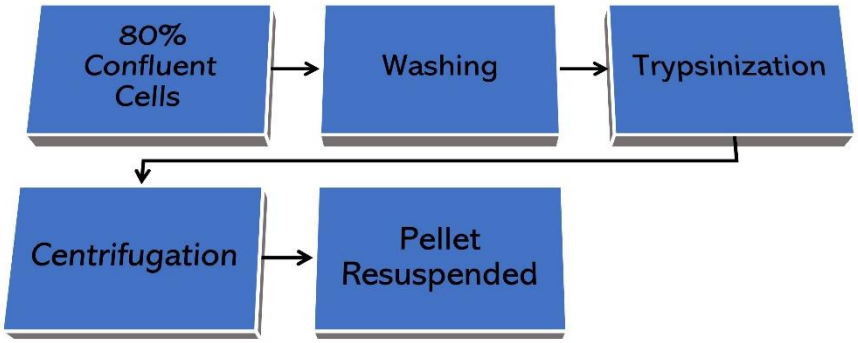
3.3.7 Cell Maintenance

Vero B4 Cells (ATCC CCL-81) were maintained in cell culture flask using DMEM with 5% FBS and 0.5% Pen Strap at 37℃ with 5% CO_2 level. Cells were regularly splitted using trypsin to break the adherence and were transferred to new flasks so that the culture remains in running.

3.3.8 Restoration of Host Cells from Cryopreservation

The cryo-vials were taken out and thawed or were put in a water bath at 27℃. 100µl of complete DMEM media was added and then the cells were then pipetted out to an Eppendorf tube which was then centrifuged for 4min at 800RMP. The supernatant was then discarded carefully and the cell pellet was resuspended in 200µl of DMEM. A new cell culture flask with 7-8ml of media was taken and then the Eppendorf DMEM having the cells was added to the new flasks. The flask was then put at 37℃ incubator with 5% CO2 level.

3.3.9 Subculturing of Host Cells



When cells grown rapidly in the cell-culture flask, they often require sub-culturing as the space in the flask in limited. For sub-culturing, the media is discarded, and the cells are washed with PBS. After washing the cells are trypsinized using trypsin so that the junction between the flask and cells is broken. The trypsin is then diluted with DMEM and the whole mixture is added to Eppendorf tube which are subjected to centrifugation for 10min at 12000 RPM. The pellet is then resuspended and then added to a new flask with fresh DMEM.

3.3.10 Viral Sampling

Covid-19 Delta samples with high titer were obtained from KRL Hospital, Islamabad and were processed in NIBGE, Faisalabad. RT-PCR verified nasopharyngeal swabs submerged in Universal Transport Media (UTM) were used as the virus source. The fabrics for testing were provided by NTU, Faisalabad.

3.3.11 Virus Propagation

Propagation of COVID-19 virus was performed in VERO B4 cells. The titer of virus stock was approximately 1 x 10^4 PFU/ml. VERO-B4 cells were seeded in a 25cm^3 flask

with DMEM/High Glucose supplemented with 10% heat-inactivated Fetal Bovine Serum and 1% Penicillin/Streptomycin. The cells were incubated at 37^{o} C and 5% CO_2 until they reached a confluency of approximately 80%. Drained the media from the flask and washed the cells with maintenance medium two times. Inoculate 1 ml of the adjusted base virus on the surface of the cultured cells in the flask and spread to the whole surface. Put the flask in a CO2 incubator (5%) at a temperature of 37 °C and keep it for 1h to absorb the virus into the cells. Add 10 ml of the maintenance medium to the flask. Placed the flask in an incubator for 5-6 days to multiply the virus. Observed the cytopathic effect through a light microscope. The cells were then detached from the growth surface with Trypsin and centrifuged at 3200 rpm (2136x g) at 4oC for 10 minutes to eliminate cell debris. The virus recovered from the supernatant contains primarily mature (infectious) COVID-19, although the various isoforms can never be isolated with 100% efficiency. The supernatant was aliquoted and stored at −80°C until use. Virus stock was filtered before use using 0.22μm filter membranes. Before use, put the cryopreserved virus suspension in the water bath at a temperature of 37 °C and kept for rapid defrosting. This was a virus suspension for the test. The concentration of the virus suspension for the test was adjusted to a titre of 1 × 105 PFU or TCID50/ml.

3.3.12 Titer Maintenance

The viral titer was maintained by TCID-50 assay (median tissue culture infectious dose), which is the viral dilution required to infect 50% of cells in the culture. Vero-B4 cells were used for the assay. Cells were grown in 75ml cell culture flask using DMEM in 5% FBS. Fully confluent flask was trypsinized and cells were counted.

3.3.12.1 Cell Counting

For cell counting trypan blue dye was mixed with media containing cells in a ratio of 1:1 and 10µl of the trypan-blue cell mixture was added to hemocytometer. Cells were counted using the grid lines observed under inverted microscope and were calculated according to the given formula.

Cells in 1ml of media = dilution factor * number of cells counted / total area counted * 10,000

3.3.12.2 TCID 50

Five thousand cells per were added to a 96-well plate incubated for 24 hours for monolayer formation. A second 96-well plate was used to prepare the viral dilutions needed for the assay. Ten replicates (horizontal) of 8 dilutions (vertical) were used, and two columns were used as a negative control. Eight dilutions of the virus-containing UTM were made from 10^{-1} up to $10^{-8,}$ and 100µl was added to the corresponding well with the cells. The wells were observed inverted daily. The viral dilution causing cell death in more than 50% of the replicates was considered vital. The viral sample's Plaque Forming Units (PFUs) were calculated using a formula. The viral sample was diluted to 10^6 PFUs for further experimentation.

3.3.13 Fabric Sample Testing

The fabric was autoclaved before experimentation at 121℃ at 15psi for 45 minutes and UV treated . Maintained cells were transferred to a 96-well plate one day before testing for monolayer formation. 2x2 square-inch pieces of fabric were cut and placed in a sterile petri dish, and 100µl of viral UTM having 10^6 PFUs was placed on the fabric to absorb. The fabric and the viral UTM were incubated for one hour. Next, the fabric was carefully taken out and was placed in 50ml falcon tubes with FBS free

DMEM, and the tube was centrifuged at 10,000 RPM for 12minutes for all the viral particles left to seep out of the fabric. 100µl DMEM from the centrifuged falcon is added to a 96-well plate with cells, apart from the negative control columns. The 96-well plate was then incubated and was observed daily under an inverted microscope for cellular damage. Spearman-karber TCID-50 equation was then applied to find comparative decrease in viral titer, and the results were expressed in terms of log viral infectivity titer.

3.3.13.1 Titer Calculation

The cytopathic effect is observed under the microscope and the wells which show cytopathic effect were noted. The TCID 50 Formula given below was used to calculate the viral titer left in the well after treatment with the fabrics.

$$\boldsymbol{logID50 = log(highest\ dilution\ giving\ 100\%\ CPE) + 0.5 - \frac{total\ wells\ showing\ CPE}{number\ of\ wells\ per\ dilution}}$$

Chapter 4

RESULTS

4.1 Antibacterial Testing

4.1.1 Characterization of Copper coated nano-fabrics through antibacterial assays

4.1.1.1 Antibacterial activity of CELD-01

(a) Qualitative Evaluation

The antibacterial activity of Copper-coated nano-fabrics was assessed using qualitative and quantitative assays to investigate the efficacy. The antibacterial activity of CELD-01 nanoparticles were tested by the agar diffusion method. Gram-negative (E.coli ATCC 8739) and gram-positive bacteria (S.aureus ATCC 6538) were used. The bacteria's primary culture was prepared in 10ml PBS solution from an overnight grown stock-streaked plate at 37 ^{0}C for 18 hours. CuO nanoparticles had an inhibitory effect against Staphylococcus aureus with an average diameter of 14.5mm.

Table 1a: Antibacterial assay by qualitative measurement of the zone of inhibition.

Sr.#	**Fabrics Treated**	**Bacterial Strain**	**Zone of Inhibition**		**Mean**	**Standard Deviation**
			1	**2**		
1	Control Fabric	*S. aureus*	0mm	0mm	0mm	0
		E.coli	0mm	0mm	0mm	0
2	CELD-01	*S. aureus*	15mm	14mm	14.5	0.5
		E.coli	12mm	11mm	11.5	0.5

(b) Quantitative Evaluation

ISO 20743was followed to assess the qualitative potential of nano-particles. Gram-negative bacteria *E.coli* (ATCC 8739) and gram-positive bacteria *S. aureus* (ATCC 6538) were used for this purpose***. Figures 4(a) & 4(b) show the number of Gram-positive and gram-negative bacterial populations*** in logarithmic value of colony-forming unit per ml (CFU/ml) after inoculating at 0hours and 24 hours. Control and treated samples were incubated for 24 hours. The number of colonies was counted to calculate the percent reduction of the bacteria by the equation given below and presented in ***Table 1b.***

Percentage mortality= (LogTt-LogCt)* 100/Lo

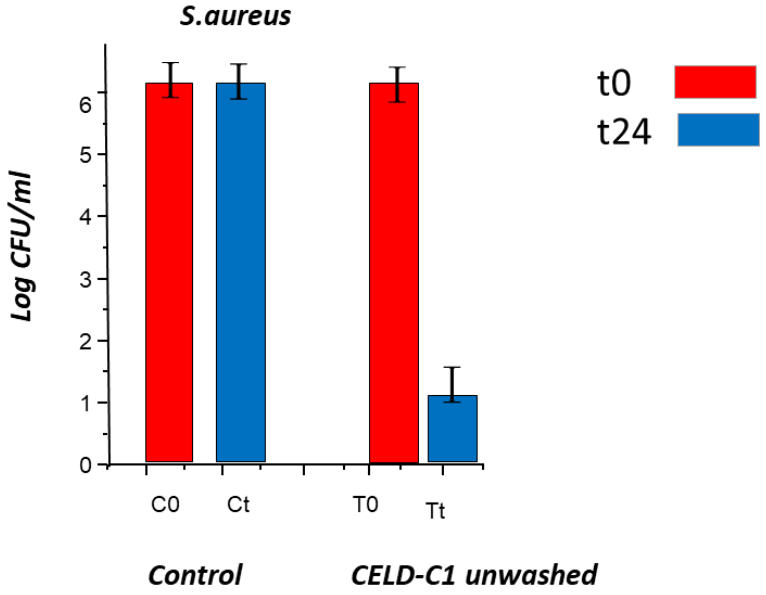


Fig 4a. Antibacterial activity of sample (**CELD-C1)**, i.e. untreated (C), Nano coated fabric (T) against *S. aureus*. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

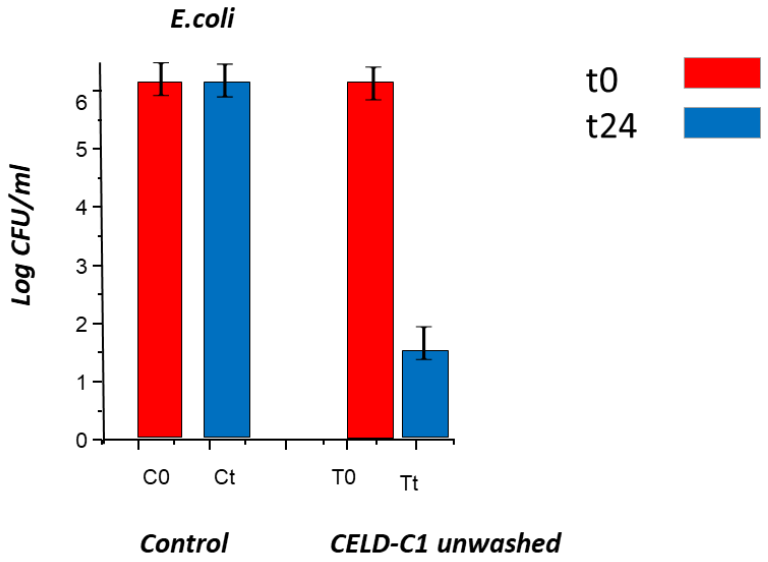


Fig 4b. Antibacterial activity of sample (CELD-C1), i.e. untreated (C), Nano coated fabric (T) against E.Coli. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

Table 1b: Percent reduction of bacteria for CELD-C1 treated sample

Bacteria	**Control**	**CELD-C1**
S. Aureus	0	99.9%
E. Coli	0	94%

4.1.1.2 Antibacterial activity of CELD-003

(a) Quantitative Evaluation

The antibacterial activity of CELD-003 nanoparticles was tested by the agar diffusion method. Gram-positive (E.coli ATCC 8739) and gram-negative bacteria (S.aureus ATCC 6538) were used. Bacterial Primary culture was prepared in 10ml PBS solution from an overnight grown stock-streaked plate at 37 ^{0}C for 18 hours.

Table 2a: Antibacterial assay by qualitative measurement of the zone of inhibition.

Sr.#	Fabrics Treated	Bacterial Strain	Zone of Inhibition 1	Zone of Inhibition 2	Mean	Standard Deviation
1	Control	*S. aureus*	0mm	0mm	0mm	0mm
		E.coli	0mm	0mm	0mm	0mm
2	CELD-003	*E. coli*	Beneath fabric	Beneath fabric	-	-

(b) Quantitative Evaluation

To carry out the quantitative evaluation of antibacterial activity, ISO 20743 was followed. Gram-positive bacteria *E.coli* (ATCC 8739) and gram-negative bacteria S. aureus (ATCC 6538) were used for this purpose***. Figures 5a & 5b show the number of Gram-positive and gram-negative bacterial populations*** in logarithmic value of colony-forming unit per ml (CFU/ml) after inoculating at 0hours and 24 hours. Control and treated samples were incubated for 24 hours. The number of colonies was counted to calculate the percent reduction of the bacteria by the equation given below and presented in ***Table 2b.***

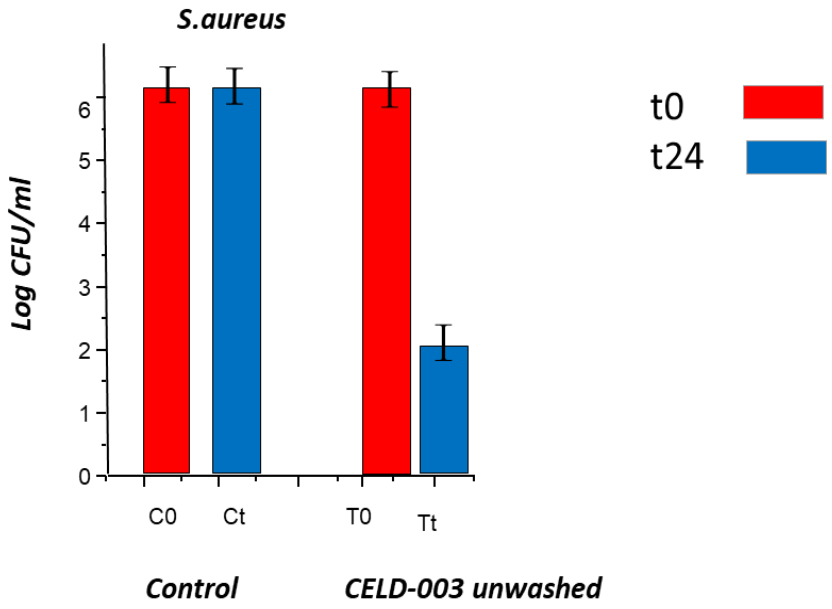


Fig 5a Antibacterial activity of sample (**CELD-003)**, i.e. untreated (C), Nano coated fabric (T) against S. aureus. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

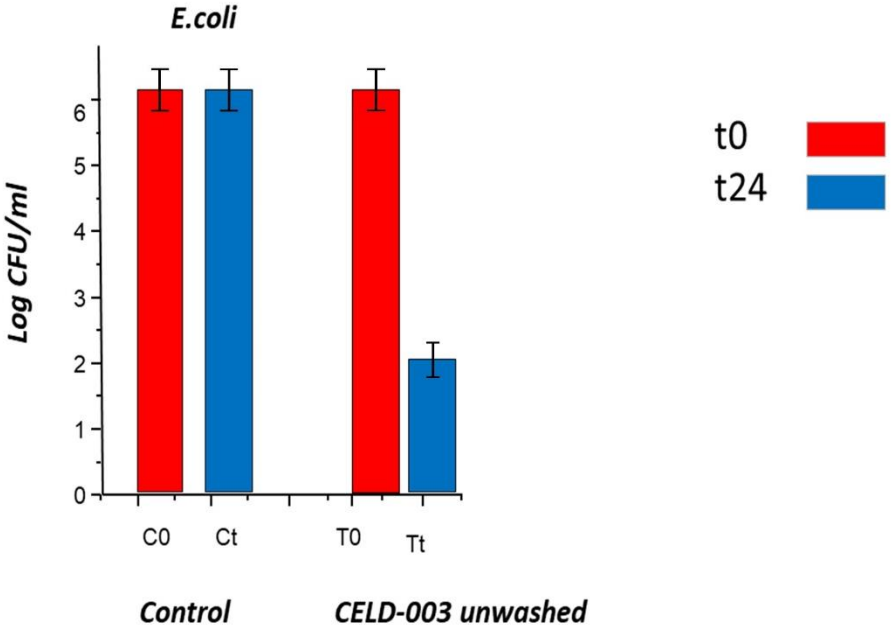


Fig 5b Antibacterial activity of sample (CELD-003), i.e. untreated (C), Nano coated fabric (T) against *E.coli*. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

Table 2b: Percent reduction of bacteria for CELD-C003 treated sample

Bacteria	**Control**	**CELD-C003**
S. Aureus	0	68%
E. Coli	0	69%

4.1.1.3 Characterization of J1-C-ELD-S1 coated nano-fabrics through antibacterial assays

(a) Qualitative Evaluation

The antibacterial activity of J1-C-ELD-S1 coated nano-fabrics was assessed using qualitative and quantitative assays to investigate the efficacy. The antibacterial activity of J1-C-ED-S1 nanoparticles was tested using the agar diffusion method. Gram-negative (E.coli ATCC 8739) and gram-positive bacteria (S.aureus ATCC 6538) were used. The primary culture of bacteria was prepared from an overnight grown stock-streaked plate at 37 ^{0}C for 18 hours. The number of bacteria was adjusted in the range of 1-3.5*10^^{6}. J1-C-ED-S1 nanoparticles had shown an inhibitory effect against Staphylococcus aureus with an average zone of inhibition of 24.5 mm.

Table 3a: Antibacterial assay by qualitative measurement of the zone of inhibition.

Sr.#	Fabrics Treated	Organisms	Diameter(mm) (cmx10=mm) 1	2	Mean	St.Dev.
01	**Control**	*S. aureus*	Zero	Zero	Zero	Zero
		E.coli	Zero	Zero	Zero	Zero
02	**J1-C-ELD-S1**	*S. aureus*	24mm	25mm	24.5mm	0.5
		E.coli	23mm	24mm	23.5mm	0.5

(b) Quantitative Evaluation

To carry out the quantitative evaluation of antibacterial activity, ISO 20743 was followed. Gram-negative bacteria E.coli (ATCC 8739) and gram-positive bacteria S. aureus (ATCC 6538) were used for this purpose**. Figures 6a & 6b show the number of Gram-positive and gram-negative bacterial populations** in logarithmic value of colony-forming unit per ml (CFU/ml) after inoculating at 0hours and 24 hours. Control and treated samples were incubated for 24 hours. The number of colonies was counted to calculate the percent reduction of the bacteria by the equation given below and presented in **Table 3b.**

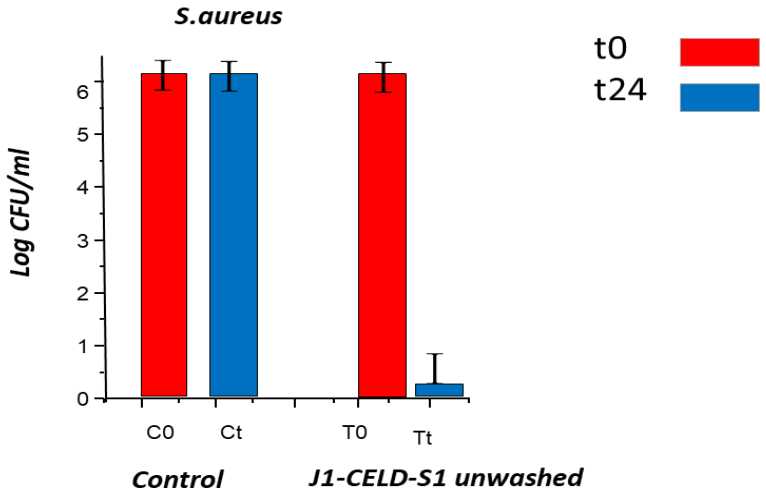


Fig 6a Antibacterial activity of sample (J1-CELD-S1), i.e. untreated (C), Nano coated fabric (T) against *S. aureus*. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

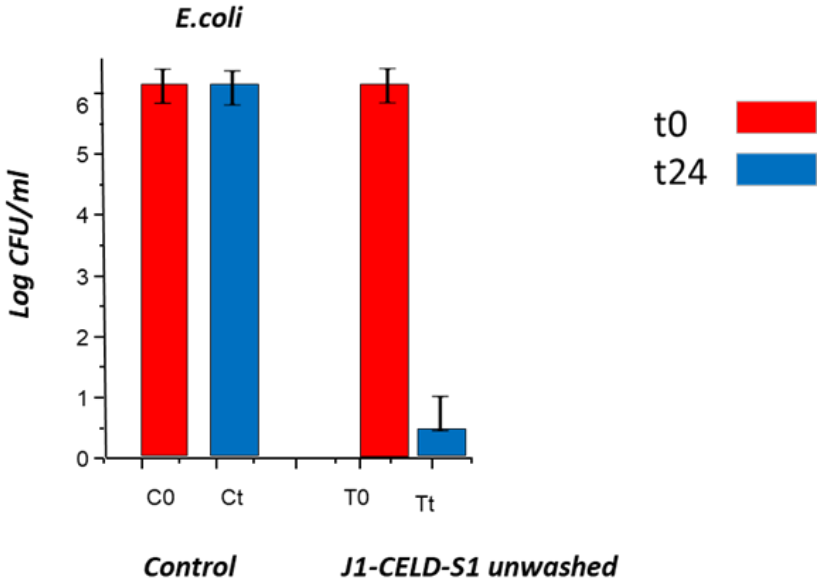


Fig 6b. Antibacterial activity of sample (**J1-CELD-S1**), i.e. untreated (C), Nano coated fabric (T) against *E.Coli*. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

Table 3b: Percent reduction of bacteria for J*1-C-ELD-S1 *treated sample

Bacteria	**Control**	***J*1-C-ELD-S1**
S. Aureus	0	94%
E. Coli	0	90%

4.1.1.4 Characterization of J1-CELD-01(D) coated nano-fabrics through antibacterial assays

(a) Qualitative Evaluation

The antibacterial activity of J1-CELD-01(D) coated nano-fabrics was assessed using qualitative and quantitative assays to investigate the efficacy. The agar diffusion method tested the antibacterial activity of J1-CELD-01(D) nanoparticles. Gram-negative (E.coli ATCC 8739) and gram-positive bacteria (S.aureus ATCC 6538) were used. Primary culture of bacteria was prepared from an overnight grown stock streaked plate at 37 ^{0}C for 18 hours.J1-CELD-01(D) nanoparticles had shown an inhibitory effect against Staphylococcus aureus with an average zone of inhibition of 25.5 mm.

Table 4a: Antibacterial assay by qualitative measurement of the zone of inhibition.

Sr.#	**Fabrics Treated**	**Bacterial Strain**	**Diameter(cm) (cmx10=mm)**		**Mean**	**Standard Deviation**
			1	**2**		
1	**Control**	*S. aureus*	Zero	0mm	0mm	0mm
		E.coli	Zero	0mm	0mm	0mm
2	**J1-CELD-01(D)**	*S. aureus*	25mm	26mm	25.5	0.5
		E.coli	20mm	20mm	20	0

(b) Quantitative Evaluation

To carry out the quantitative evaluation of antibacterial activity, ISO 20743 was followed. Gram-negative bacteria E.coli (ATCC 8739) and gram-positive bacteria S. aureus (ATCC 6538) were used for this purpose***. Figures 7a & 7b show the number of Gram-positive and gram-negative bacterial populations*** in logarithmic value of colony-forming unit per ml (CFU/ml) after inoculating at 0hours and 24 hours. Control and treated samples were incubated for 24 hours. The number of colonies was counted to calculate the percent reduction of the bacteria by the equation given below and presented in ***Table 4b.***

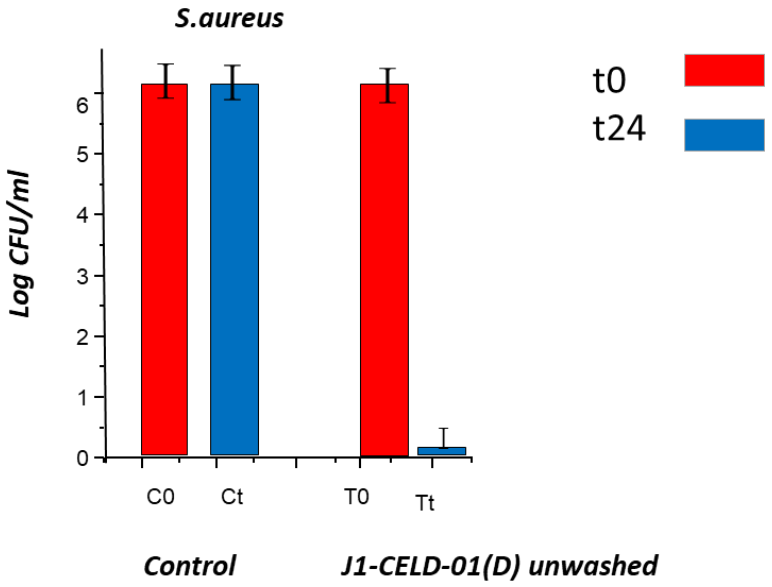


Fig 7a Antibacterial activity of sample (J1-CELD-01(D)), i.e. untreated (C), Nano coated fabric (T) against *S. aureus*. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

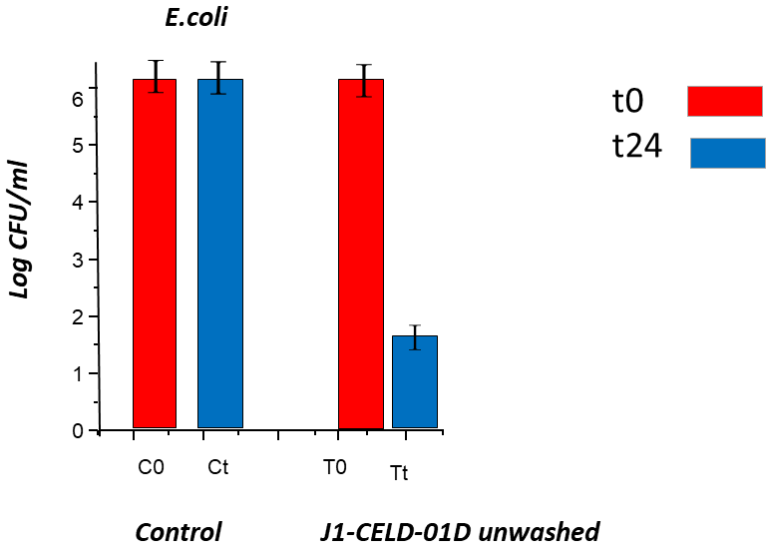


Fig 7b Antibacterial activity of sample (**J1-CELD-01D**), i.e. untreated (C), Nano coated fabric (T) against *E.Coli*. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

Table 4b: Percent reduction of bacteria for J1-CELD-01(D) treated sample

Bacteria	**Control**	***J1-CELD-01(D)***
S. Aureus	0	99.5%
E. Coli	0	76%

4.1.1.5 Characterization of CQD-C1 coated nano-fabrics through antibacterial assays

(a) Qualitative Evaluation

The antibacterial activity of CQD-C1 coated nano-fabrics was assessed using qualitative and quantitative assays to investigate the efficacy. The antibacterial ability of CQD-C1 nanoparticles was tested using the agar diffusion method. Gram-negative (E. coli ATCC 8739) and gram-positive bacteria (S. aureus ATCC 6538) were used for this purpose. The primary culture of bacteria was prepared from an overnight grown stock-streaked plate at 37 ^{0}C for 18 hours. The number of bacteria was adjusted in the range of 1-3.5*10^^{6}. CQD-C1 nanoparticles had shown the most significant inhibitory effect against Staphylococcus aureus with an average zone of inhibition of 32.5 mm diameter.

Table 5a: Antibacterial assay by qualitative measurement of the zone of inhibition.

Sr.#	Fabrics Treated	Bacterial Strains	Diameter 1	Diameter 2	Mean	St.Dev.
01	**Control**	*S. aureus*	0mm	0mm	0mm	0mm
		E.coli	0mm	0mm	0mm	0mm
02	**CQD-C1**	*S.aureus*	32mm	33mm	32.5	0.5
		E.coli	20mm	20mm	20	0.5

3.13.2 Quantitative Evaluation

To carry out the quantitative evaluation of antibacterial activity, ISO 20743 was followed. Gram-positive bacteria E.coli (ATCC 8739) and gram-negative bacteria S. aureus (ATCC 6538) were used for this purpose**. *Figures 8a & 8b show the number of Gram-positive and gram-negative bacterial populations*** in logarithmic value of colony-forming unit per ml (CFU/ml) after inoculating at 0hours and 24 hours. Control and treated samples were incubated for 24 hours. The number of colonies was counted to calculate the percent reduction of the bacteria by the equation given below and presented in ***Table 5b***

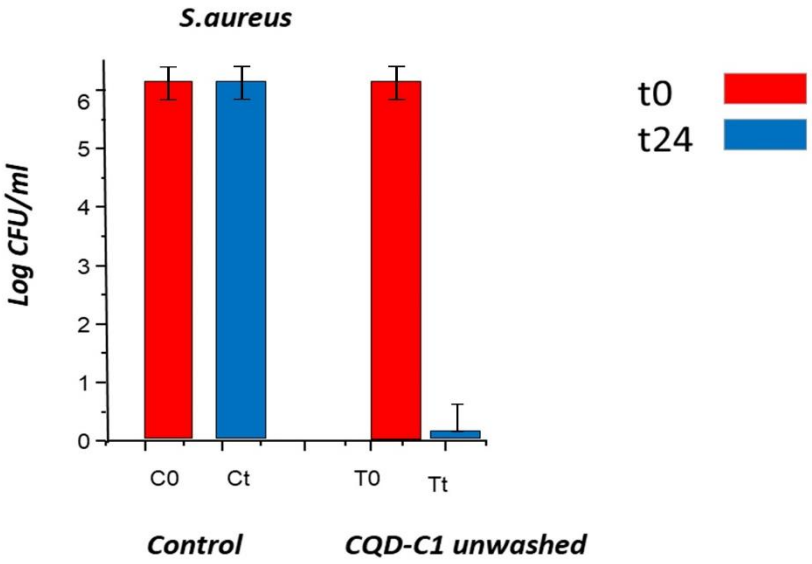


Fig 8a: Antibacterial activity of sample (**CQD-C1**), i.e. untreated (C), Nano coated fabric (T) against *S. aureus*. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

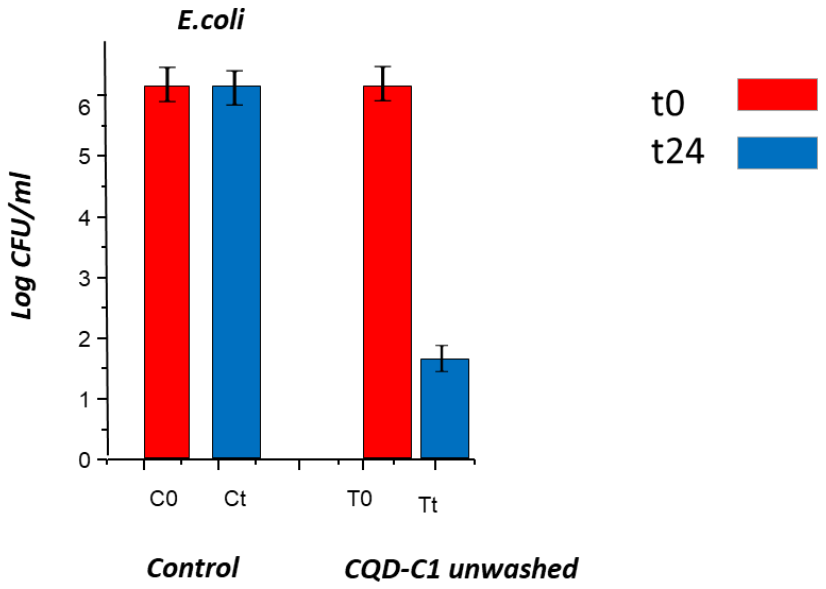


Fig 8b: Antibacterial activity of sample (**CQD-C1**), i.e. untreated (C), Nano coated fabric (T) against *E.coli*. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

Table 5b: Percent reduction of bacteria for* CQD-C1 *treated sample

Bacteria	**Control**	**CQD-C1**
S. Aureus	0	99.5%
E. Coli	0	70%

4.1.1.6 Characterization of GL-06 coated nano-fabrics through antibacterial assays.

(a) Qualitative Evaluation:

The antibacterial activity of GL-06 coated nano-fabrics was assessed using qualitative and quantitative assays to investigate the efficacy under different conditions. The agar diffusion method tested the antibacterial activity of GL-06 nanoparticles, and Gram-negative (E.coli ATCC 8739) and gram-positive bacteria (S.aureus ATCC 6538) were used. The primary culture of bacteria was prepared from an overnight grown stock-streaked plate at 37 ^{0}C for 18 hours. GL-06 nanoparticles had shown antibacterial activity beneath of fabric patch because of the reason that subunits are bonded/locked to each other.

Table 6a: Antibacterial assay by qualitative measurement of the zone of inhibition.

Sr.#	Fabrics Treated	Organisms	Diameter(cm) (cmx10=mm) 1	2	Mean	St.Dev.
1	**Control**	S. aureus	0mm	0mm	0mm	0mm
		E.coli	0mm	0mm	0mm	0mm
2	**GL-06**	S. aureus (beneath fabric)	20mm	20mm	20	0
		E.coli	20mm	20mm	20	0

(b) Quantitative Evaluation

To carry out the quantitative evaluation of antibacterial activity, ISO 20743 was followed. Gram-negative bacteria E.coli (ATCC 8739) and gram-positive bacteria S. aureus (ATCC 6538) were used for this purpose**. Figures 9a & 9b show the number of Gram-positive and gram-negative bacterial populations** in logarithmic value of colony-forming unit per ml (CFU/ml) after inoculating at 0hours and 24 hours. Control and treated samples were incubated for 24 hours. The number of colonies was counted to calculate the percent reduction of the bacteria by the equation given below and presented in **Table 6b.**

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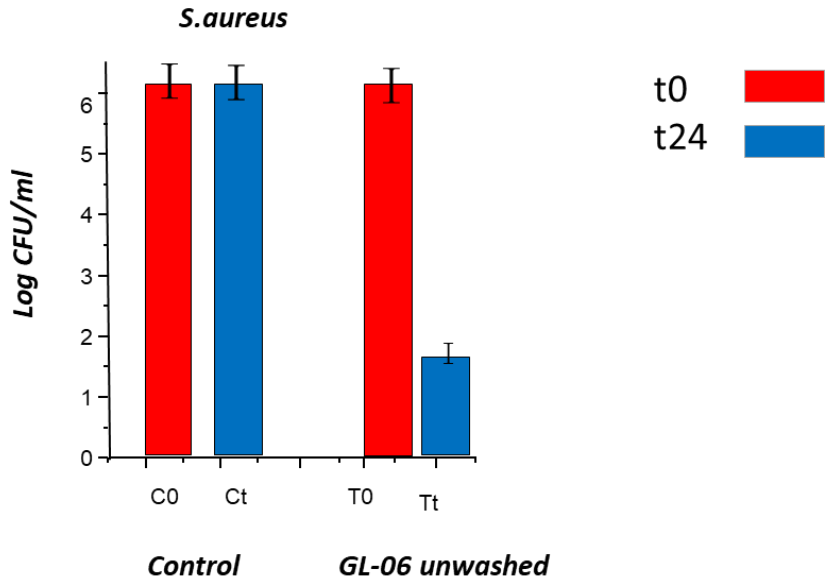


Fig 9a: Antibacterial activity of sample (**GL-06**), i.e. untreated (C), Nano coated fabric (T) against S. aureus. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

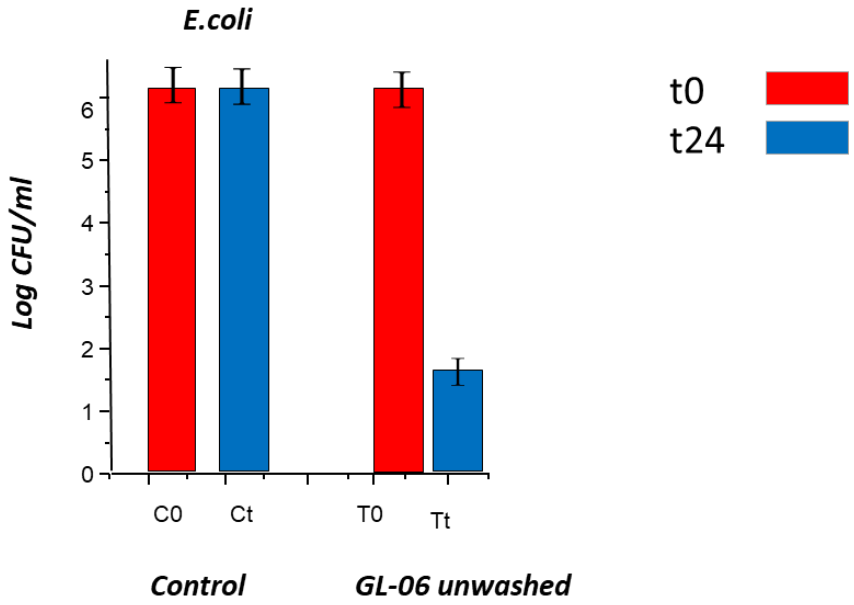


Fig 9b Antibacterial activity of sample (**GL-06**), i.e. untreated (C), Nano coated fabric (T) against E.coli. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

Table 6b: Percent reduction of bacteria for* GL-06 *treated sample

Bacteria	Control	GL-06
S. Aureus	0	78%
E. Coli	0	74%

4.1.2 Characterization of Titanium coated nano-fabrics through antibacterial assays.

4.1.2.1 Characterization of TiO2+GTPs + ODS coated nano-fabrics through antibacterial assays.

(a) Qualitative Evaluation

The antibacterial activity of Ti coated nano-fabrics was assessed using qualitative and quantitative assays to investigate the efficacy. The agar diffusion method tested the antibacterial activity of TiO2+GTPs + ODS nanoparticles. Gram-negative (E.coli ATCC 8739) and gram-positive bacteria (S.aureus ATCC 6538) were used. The bacteria's primary culture was prepared in 10ml PBS solution from an overnight grown stock-streaked plate at 37 ^{0}C for 18 hours. TiO2 nanoparticles had shown no inhibitory effect against E.coli.

Table 7a: Antibacterial assay by qualitative measurement of the zone of inhibition.

Sr.#	Fabrics Treated	Organisms	Zone of Inhibition 1	Zone of Inhibition 2	Mean	St.Dev
1	Control	S. aureus	zero	zero	zero	zero
		E.coli	zero	zero	zero	zero
2	TiO2+GTPs + ODS	E. coli	0	0	0	0

(b) Quantitative Evaluation

To carry out the quantitative evaluation of antibacterial activity, ISO 20743 was followed. Gram-positive bacteria E.coli (ATCC 8739) and gram-negative bacteria S. aureus (ATCC 6538) were used for this purpose**. Figures 10a & 10b show the number of Gram-positive and gram-negative bacterial populations** in logarithmic value of colony-forming unit per ml (CFU/ml) after inoculating at 0hours and 24 hours. Control and treated samples were incubated for 24 hours. The number of colonies was counted to calculate the percent reduction of the bacteria by the equation given below and presented in **Table 2.**

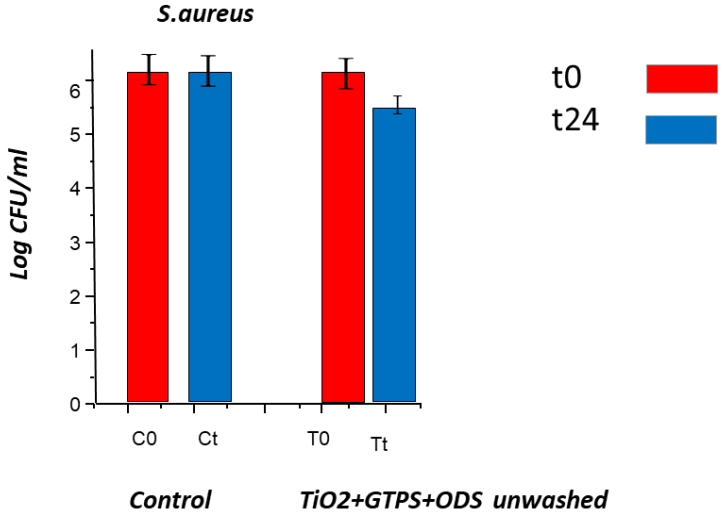


Fig 10a Antibacterial activity of sample (**TiO2+GTPS+ODS**), i.e. untreated (C), Nano coated fabric (T) against *S. aureus*. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

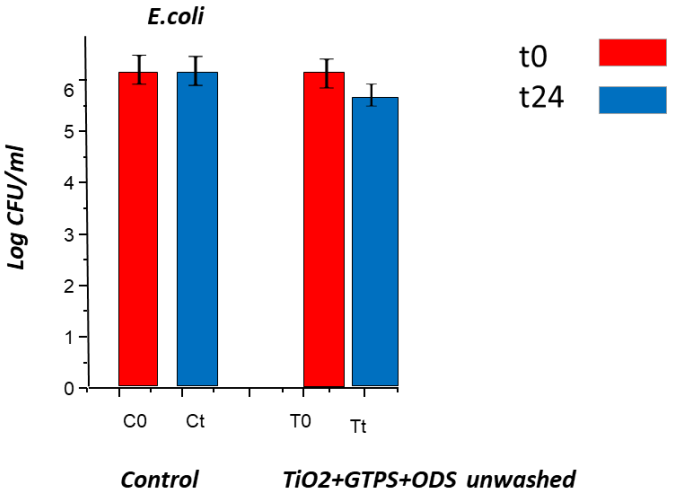


Fig 10b: Antibacterial activity of sample (**TiO2+GTPS+ODS**), i.e. untreated (C), Nano coated fabric (T) against *E.Coli*. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

Table 7b: Percent reduction of bacteria for* TiO2+GTPS+ODS *treated sample

Bacteria	**Control**	**TiO2+GTPS+ODS**
S. Aureus	0	10%
E. Coli	0	9%

4.1.2.2 Characterization of R.Ti+G6 coated nano-fabrics through antibacterial assays.

(a) Qualitative Evaluation

The antibacterial activity of R.Ti+G6 coated nano-fabrics was assessed using qualitative and quantitative assays to investigate the efficacy. The antibacterial activity of R.Ti+G6 nanoparticles was tested by the agar diffusion method. Gram-negative (E.coli ATCC 8739) and gram-positive bacteria (S.aureus ATCC 6538) were used. The bacteria's primary culture was prepared in 10ml PBS solution from an overnight grown stock-streaked plate at 37 ^{0}C for 18 hours.

Table 8a: Antibacterial assay by qualitative measurement of the zone of inhibition.

Sr.#	**Fabrics Treated**	**Organisms**	**Diameter(cm) (cmx10=mm)**		**Mean**	**St.Dev.**
			1	**2**		
1	**Control**	S. aureus	Zero	Zero	Zero	Zero
		E.coli	Zero	Zero	Zero	Zero
2	**R.Ti+G6**	S.aureus	0mm	0mm	0	0
		E.coli	0	0	0	0

(b) Quantitative Evaluation

To carry out the quantitative evaluation of antibacterial activity, ISO 20743 was followed. Gram-negative bacteria E.coli (ATCC 8739) and gram-positive bacteria S. aureus (ATCC 6538) were used for this purpose**. *Figures 11a & 11b show the number of Gram-positive and gram-negative bacterial populations*** in logarithmic value of colony-forming unit

per ml (CFU/ml) after inoculating at 0hours and 24 hours. Control and treated samples were incubated for 24 hours. The number of colonies was counted to calculate the percent reduction of the bacteria by the equation given below and presented in ***Table 8b.***

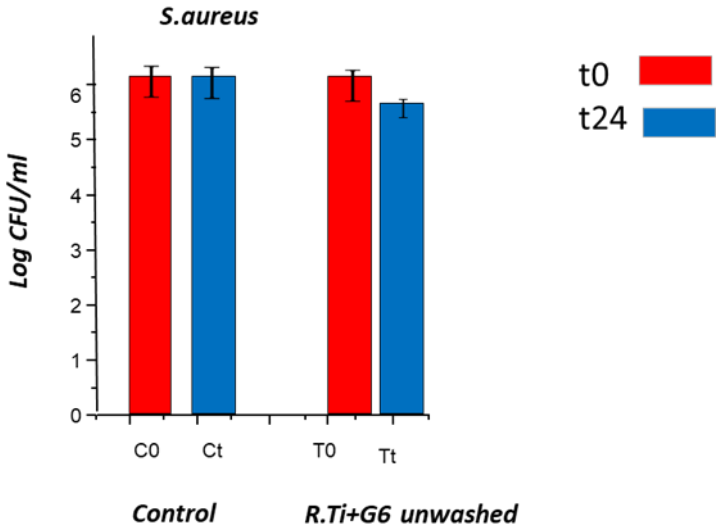


Fig 11a. Antibacterial activity of sample (**R.Ti+G6**), i.e. untreated (C), Nano coated fabric (T) against *S. aureus*. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

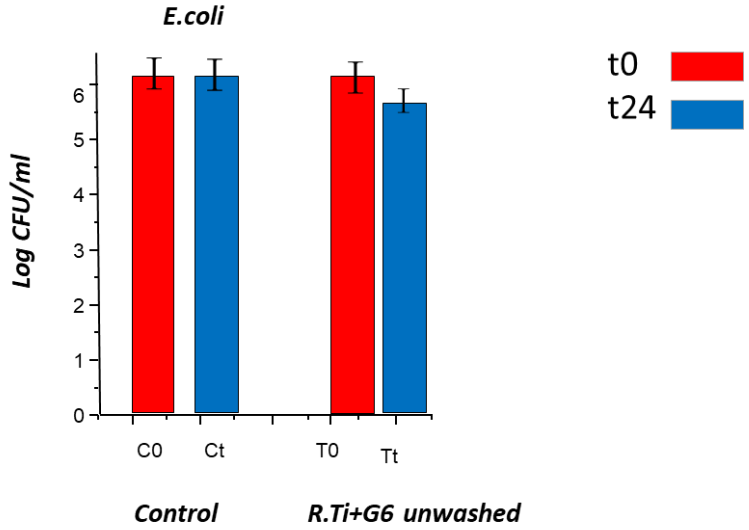


Fig 11b. Antibacterial activity of sample (**R.Ti+G6**), i.e. untreated (C), Nano coated fabric (T) against.*E.coli*. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

Table 8b: Percent reduction of bacteria for R.Ti+G6 treated sample

Bacteria	**Control**	***R.Ti+G6***
S. Aureus	0	9%
E. Coli	0	10%

4.1.2.3 Characterization of TOSAC coated nano-fabrics through antibacterial assays.

(a) Qualitative Evaluation

TOSAC coated nano-fabrics' antibacterial activity was assessed using qualitative and quantitative assays to investigate the efficacy. The antibacterial activity of TOSAC nanoparticles was tested by the agar diffusion method. Gram-negative (E.coli ATCC 8739) and gram-positive bacteria (S.aureus ATCC 6538) were used. The bacteria's primary culture was prepared in 10ml PBS solution from an overnight grown stock-streaked plate at 37 ^{0}C for 18 hours.

Table 9a: Antibacterial assay by qualitative measurement of the zone of inhibition.

Sr.#	**Fabrics Treated**	**Organisms**	**Diameter(cm) (cmx10=mm)**		**Mean**	**St.Dev.**
			1	**2**		
1	**Control**	S. aureus	Zero	Zero	Zero	Zero
		E.coli	Zero	Zero	Zero	Zero
2	**TOSAC-01**	S.aureus	35mm	36mm	35.5	0.5
		E.coli	27mm	28mm	27.5mm	0.5

(b) Quantitative Evaluation

To carry out the quantitative evaluation of antibacterial activity, ISO 20743 was followed. Gram-negative bacteria E.coli (ATCC 8739) and gram-positive bacteria S. aureus (ATCC 6538) were used for this purpose***. Figures 12a & 12b show the number of Gram-positive and gram-negative bacterial populations*** in logarithmic value of colony-forming unit per ml (CFU/ml) after inoculating at 0hours and 24 hours. Control and treated samples were incubated for 24 hours. The number of colonies was counted to calculate the percent reduction of the bacteria by the equation given below and presented in ***Table 9b.***

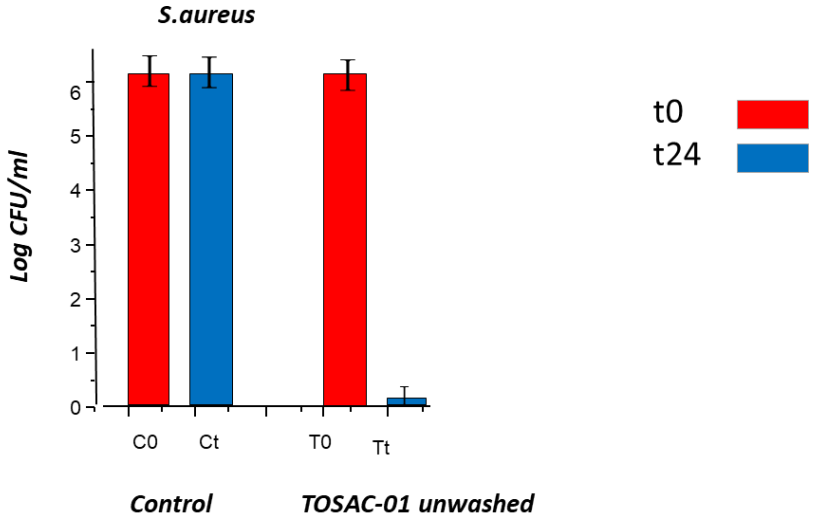


Fig 12a Antibacterial activity of sample (**TOSAC-01**), i.e. untreated (C), Nano coated fabric (T) against S. aureus. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

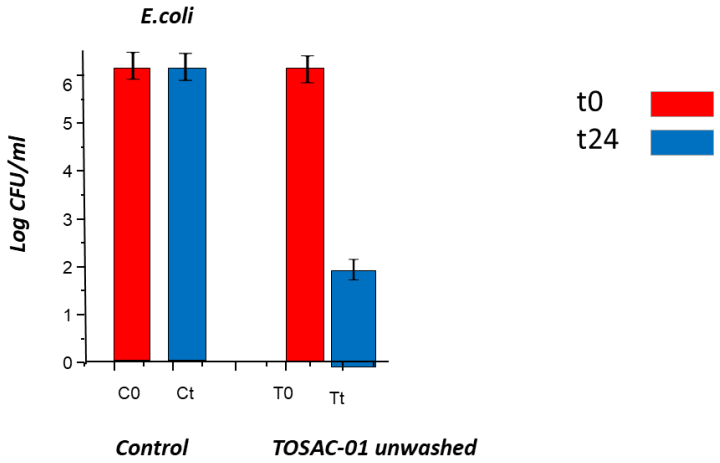


Fig 12b Antibacterial activity of sample (**TOSAC-01**), i.e. untreated (C), Nano coated fabric (T) against E.Coli. All experiments were run in triplicate and graph were generated by using Origin Pro 8.5.

Table 9b: Percent reduction of bacteria for TOSAC-01 treated sample

Bacteria	**Control**	***TOSAC-01***
S. Aureus	0	99.9%
E. Coli	0	80%

4.2 Antiviral Testing

The UTM from the nasopharyngeal swab of COVID-19 positive patient (Delta variant) was used as the virus source for antiviral testing. The assay was carried out as explained in the methodology section, and the Spearman-Karber TCID-50 equation was used to calculate the decrease in viral titer after being in contact with the fabric. For this, the 96-well plate was observed daily, and the endpoint was when more than 50% of the wells showed cytopathic effect due to virus. After that , the equation was applied. The viral titer was calculated. A

percentage decrease in the viral titer was calculated compared to the maintained viral titer added initially at the start of the assay. The Spearman-Karber equation is given as:

$$\boldsymbol{logID50 = log(highest\ dilution\ giving\ 100\%\ CPE) + 0.5 - \frac{total\ wells\ showing\ CPE}{number\ of\ wells\ per\ dilution}}$$

4.2.1 Antiviral activity against Ag-coated fabrics

The antiviral activity against Ag-coated fabric (washed and unwashed) was performed at 0 hours and after 60 minutes. The percentage reduction in viral load is presented in the graph

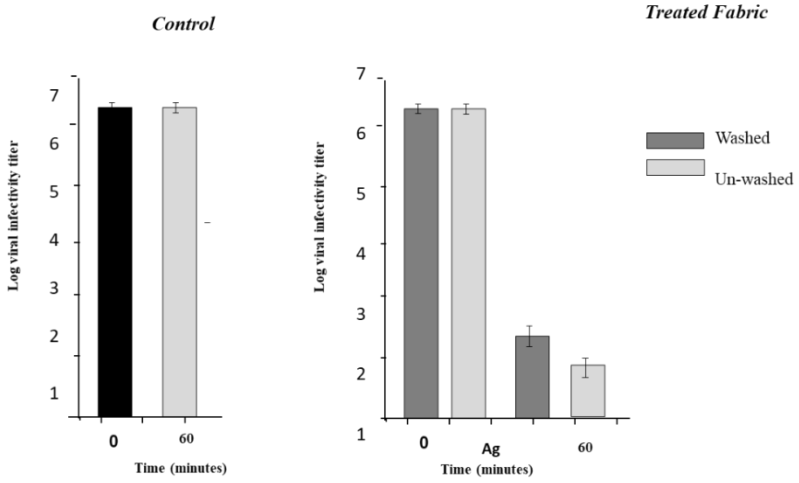


Fig 13a: Corona residual viral load determined by TCID50 on Ag-coated fabric after a contact time of 0hour & 60 minutes.; error bars are the standard error of the mean

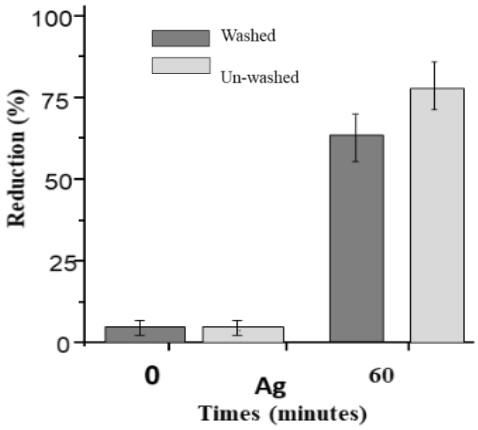


Fig13b: Comparison of % reduction in viral load of washed vs unwashed Ag-coated fabric at both 0h and 1h time.

4.2.2 Antiviral activity against Q1 & Q10-coated fabrics

The antiviral activity against Q1, Q10 -coated fabric (washed and unwashed) was performed at 0 hours and after 60 minutes. The percentage reduction in viral load is presented in the graph

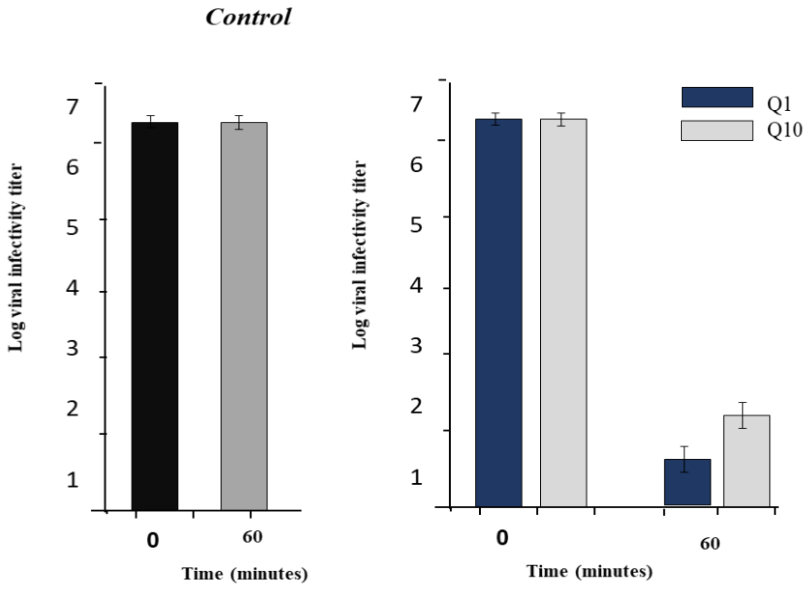


Fig 14a :Corona residual viral load determined by TCID50 on Nano coated fabrics after a contact time of 0hour, 60 minutes.

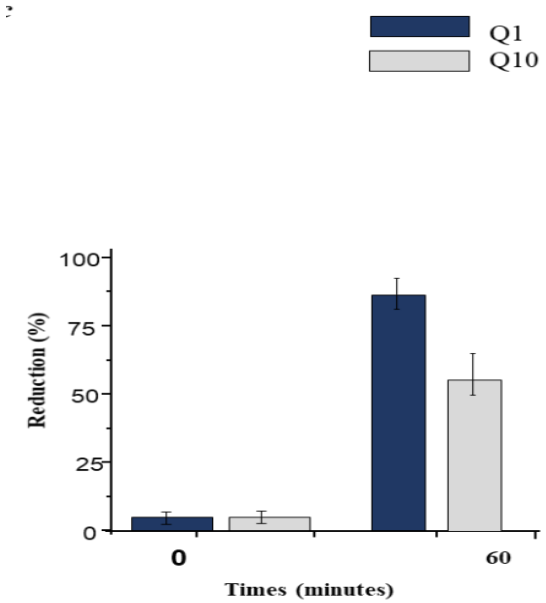


Fig 14b: Comparison of % reduction in viral load of Q1, Q10-coated fabric at both 0h and 1h time.

4.2.3 Antiviral activity against ZF & ZF1 coated fabrics

The antiviral activity against ZF, ZF1 coated fabric (washed and unwashed) was performed at 0 hours and after 60 minutes. The percentage reduction in viral load is presented in the graph

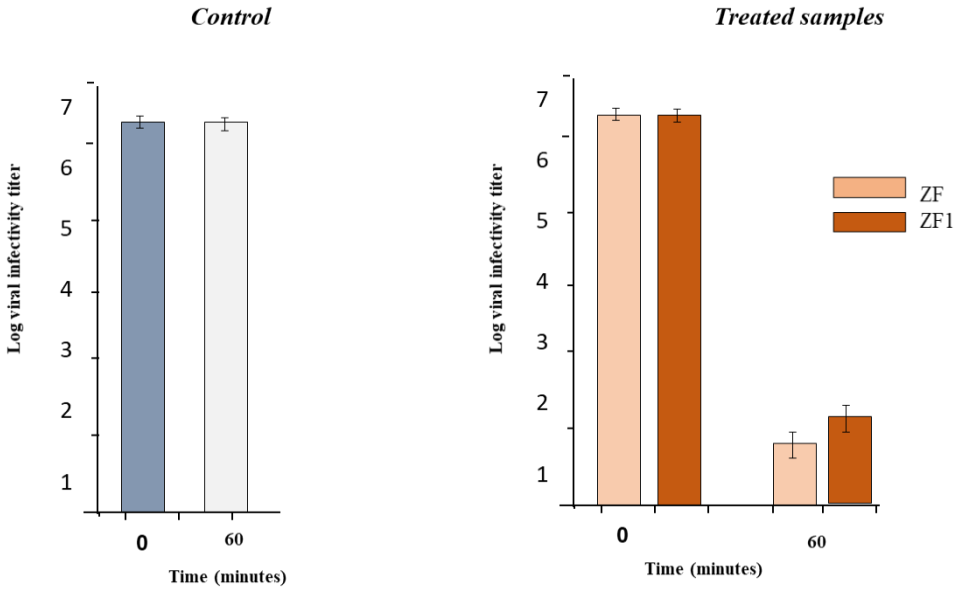


Fig 15a: Corona residual viral load determined by TCID50 on ZF , ZF1fabrics after a contact time of 0hour, 60 minutes.

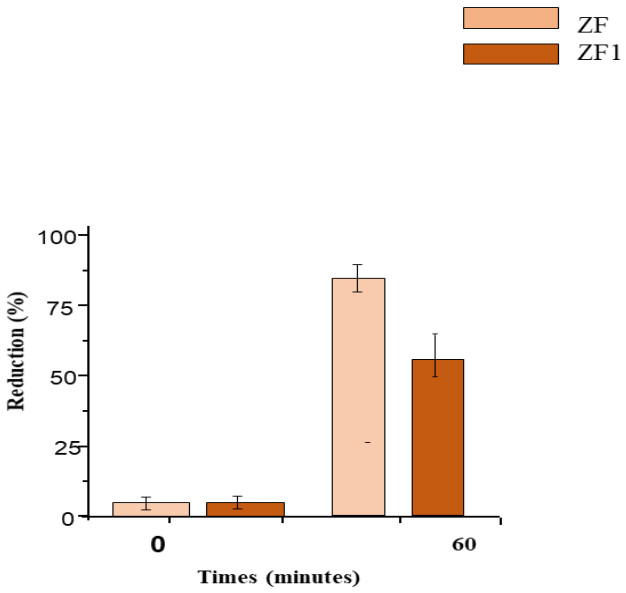


Fig 15b : Comparison of % reduction in viral load of ZF, ZF1-coated fabric at both 0h and 1h time.

4.2.4 Antiviral activity against CCPE-A, B coated fabrics

The antiviral activity against CCPEA, B coated fabric (washed and unwashed) was performed at 0 hours and after 60 minutes. The percentage reduction in viral load is presented in the graph

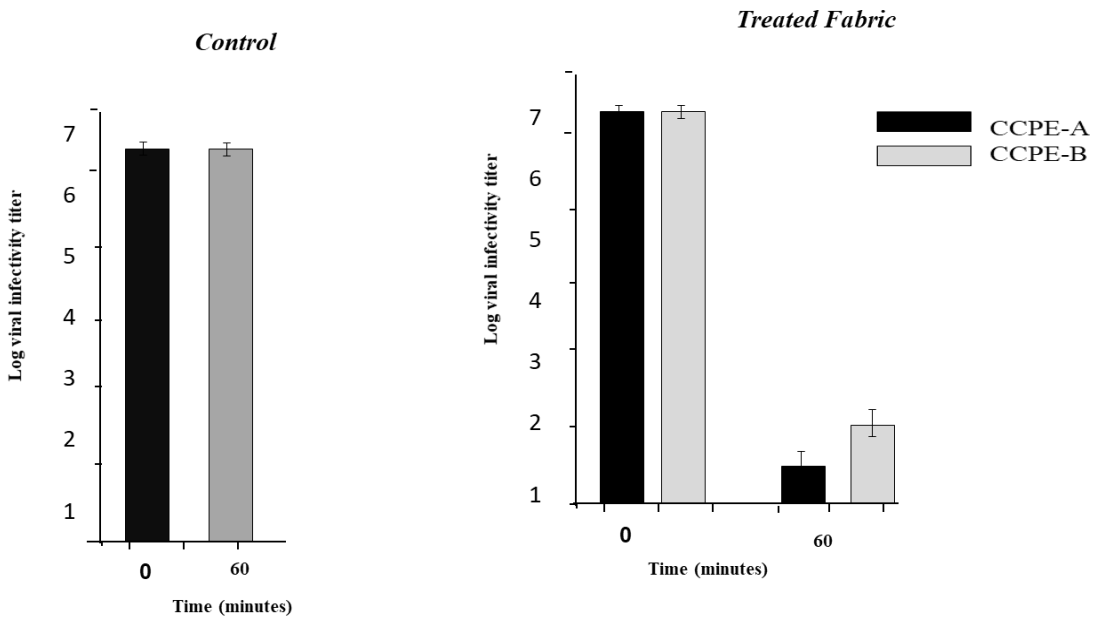


Fig 16a: Corona residual viral load determined by TCID50 on CCPEA, B fabrics after a contact time of 0hour, 60 minutes.

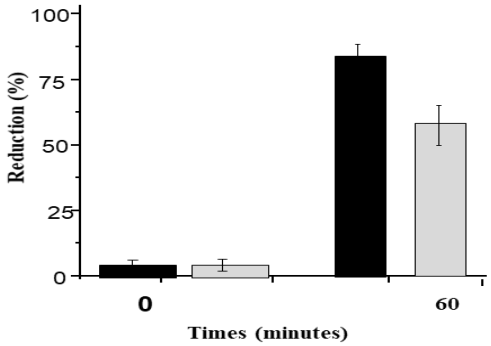


Fig 16b: Comparison of % reduction in viral load of CCPEA , B coated fabric at both 0h and 1h time.

Chapter 5

Discussion

The globe is currently in a dangerous situation owing to microbial infections. These infections are widespread, affecting global health. In Pakistan, poor health conditions are increasing illness rates. Frontline workers must do a lot to manage sickness. They may also be exposed to viruses, germs, and fungus directly or via aerosols, increasing their risk of infection. Infection-free personal protective equipment (PPE) might be designated to avoid microbial contamination.

COVID-19 epidemic has generated awareness to ensure best preventive measures to avoid its widespread. Indeed the global demand for antimicrobial materials is growing because of the increase in infections caused by bacteria and viruses such as SARS-CoV-2ng. New technologies by using polymeric systems are of great interest. Today, the need for environment-friendly antimicrobial fabrics and textiles is growing. To reduce contamination caused by nondegradable plastics , an antimicrobial cellulose fabric containing Ag nanoparticles were prepared in a study. Cellulose fabrics are used in many industries, including medical textiles, and are also widely used to prepare personal protective equipment during the COVID-19 period (Mallakpour, Azadi, & Hussain, 2021). After generating mechanoradicals, the solution's metal ions (Au3+ and Ag+ ions) were reduced to metal nanoparticles. Finally, metal nanoparticles were decorated on fabrics. The antimicrobial performance of the prepared fabric toward E. coli (Gram-negative bacteria) and Bacillus subtilis (Gram-positive bacteria) was tested, and the outcomes showed the toxicity of nanocomposite toward bacteria (Kwiczak-Yiğitbaşı et al., 2020). The silver, copper, and titanium nanocoated fabric compositions were examined. Although metal based nano coatings have shown activity in preventing and killing bacteria and viruses but its challenging to deposit them due to absence of functional groups. There is a need to immobilize with high durability, activity and scalability. Fiber reactive organic chemicals are also being used as comprising good fixation, non-hazardous nature and tunable functionalities but their potential of application in bioactive textiles is still untapped. Therefore,

they have been synthesized and their antimicrobial activity was achieved. After optimization, 50-100ul bacterial suspension was utilized. Most nanocoated textiles have similar effects. Several nano-particle-coated textiles have shown excellent antiviral activity. The antibacterial standard for testing is 10ug of Gentamycin on disc. The zone of inhibition for Staphylococcus species and E.coli is defined as 15mm for S.aureus and E.coli, respectively. A resistant microbial strain has a 12 mm inhibitory zone. The 13-14mm zone of inhibition is considered intermediate resistance to nanocoated cloth (Jan Hudzicki, 2009). The percentage decrease varied depending on the concentration of nanoparticles deposited on the cloth. Rouchi Roy (2014) claims that leaking CnO nanocoatings are 99 percent bactericidal (not bacteriostatic) against S. aureus and E. coli. CnO nanoparticles generate reactive oxygen species, increasing membrane lipid peroxidation. Sugars, DNA, and proteins seep through the membrane, compromising cell viability

Copper has been validated as a strong antibacterial agent previously (Rafique et al., 2017). Studies have suggested that the direct antibacterial effect of copper is comparatively less as compared to a bio-leaching state of copper (Bastos et al., 2020). The results of this study show promising results when copper based nanoparticles were tested. **CELD-01** fabric showed a fair zone of inhibition i.e. 14.5mm for *S.aureus* and 11.5mm for *E.coli* which indicates the bio-leaching ability of the fabric. In quantitative analysis, high efficacy of the fabric was observed as it showed approximately absolute bacterial reduction for *S.aureus* and 94% colony reduction for *E.coli*. **CELD-003** showed bacteriostatic effect in quantitative analysis but no zone of inhibition suggested that the fabric had no bio-leaching quality. When liquid inoculum was used during quantitative analysis, satisfactory results were observed after 24hours of contact time. **J1-C-ELD-S1 & J1-CELD-01(D)** fabrics showed exceptional results in quantitative as well as qualitative analysis. **CQD-C1** was more efficient against gram-positive bacteria as compared to gram-negative bacteria.

Titanium nano-particles are not commonly used for antibacterial activity apart from water-purification systems due to the reason that they are quite robust and intact in their built which ensure durability of the bioactive systems. Very low bacterial colony reduction was shown by titanium nanocoated fabrics apart from **TOSAC-01,** which showed a percent reduction of 99.99% against gram-positive and 80% against gram-negative bacteria. The light-activated Titanium fabric has shown good benefits against S.aureus and E.coli , according to Ismail R.A. et al. (2015) and Sergey V. G. et al. (2021). Compared to ZnO nanoparticles, iron oxide nanoparticles have bacteriostatic and bacteriocidal antimicrobial effects. Direct membrane binding, ROS generation, DNA damage, lipid peroxidation, protein degradation, and antibiofilm activity (Gudkov, S.V. et al., 2021). However, when the silver, titanium, and copper-based bioactive fabrics were subjected to prior light studies, they showed improved outcomes in the presence of light, demonstrating that these materials are photodynamic Due to the possibility of the coating being removed from the fabric, such as in the case of CuO, fabrics with leaching nano-coatings do not perform better after multiple items of washing . After repeated washings, it loses its antibacterial action. (Gulati, 2021) Patches of microbial development beneath fabric may be attributed to low nanocoated material concentration. The absence of microbial growth beneath the control cloth is a favorable sign of antimicrobial efficacy. The microbial growth in the form of patches beneath fabric may be due to the low concentration of nanocoated material on fabric. The control fabric has shown no zone of inhibition with microbial growth beneath the fabric; therefore, it is a positive indicator of antimicrobial effectwhen there is no growth beneath the fabric.

A limitation of the study was to accurately differentiate between the biostatic and biocidal effect of the fabrics. Some estimate could be made by comparing the results of qualitative and quantitative analysis but further validation of the fabrics is required.

TCID50 procedure was used to quantify the results from 96-well plates and the Spearman-Karber equation was used for calculation of the viral-titer. The equation goes as

$$\boldsymbol{log\ 50\%\ endpoint = x + d\ (m + l\ /2 - S)}$$

where

X is log10 of the lowest dilution

d is log 10 of the dilution factor

m is one less than the number of dilutions used

S is the sum of proportion of wells infected

The method works on finding the dilution which has the infected number of wells above and closest to 50% of wells. This method gives us the viral titer that we have left for a specific sample. Higher number of replicates ensure better and more accurate results using this formula, hence 8 technical replicates were used each time and the experiment was repeated three times . Next, the antiviral activity value for a fabric was calculated by the formula

$$\boldsymbol{Mv = lg(Va/Vc) = lg(Va) - lg(Vc)}$$

Mv is the antiviral activity value;

lg(Va) is the initial viral titer added for the assay

lg(Vc) is the common logarithm average of 3 infectivity titre values after 2 h contacting with the antiviral fabric specimen.

The **Ag-coated** nano-fabric showed satisfactory but not absolute reduction in the viral titer. The viral titer value was reduced to $10^{2.5}$ from a value of $10^{7.4}$. **Q1 & Q10** fabrics showed very good viral reduction i.e., up to $10^{1.5}$ & $10^{2.1}$ respectively. **ZF & ZF1** nano-coated fabrics showed a percentage reduction of about 86% and 62%. Similarly, **CCPE-A & CCPE-B** fabrics showed promising results with 85% and 65% reduction in viral titer after 60 minutes of contact time. CCPE is a fiber reactive organic chemical with two free OH group. It was prepared by chemical method and deposited by pad-dry-cure method. Initial interaction between quaternary ammonium salts and viral envelope is reported to be a result of electrostatic interaction between positively charged quaternary ammonium salt and the negatively charged microbial membranes .The antiviral effect of AgNPs covered fabric is mediated by reactive oxygen species, lipid peroxidation, and free radicals that induce protein and nucleic acid denaturation and damage the proton pump and cell wall. They thereby change membrane permeability and cell survival (Qian, Y. et al., 2013; Rai M. et al., 2021). Silver nanoparticle concentration affects antiviral activity (Prittesh, K. et al., 2018; Ediz, E. et al., 2020). In another study , the silver's potential against COVID-19 as a disinfectant was studied and encouraging results were found as the silver possibly showed hurdles in viral attachment, breaking the di-sulfide bridges necessary for attachment (Behbudi, 2021).

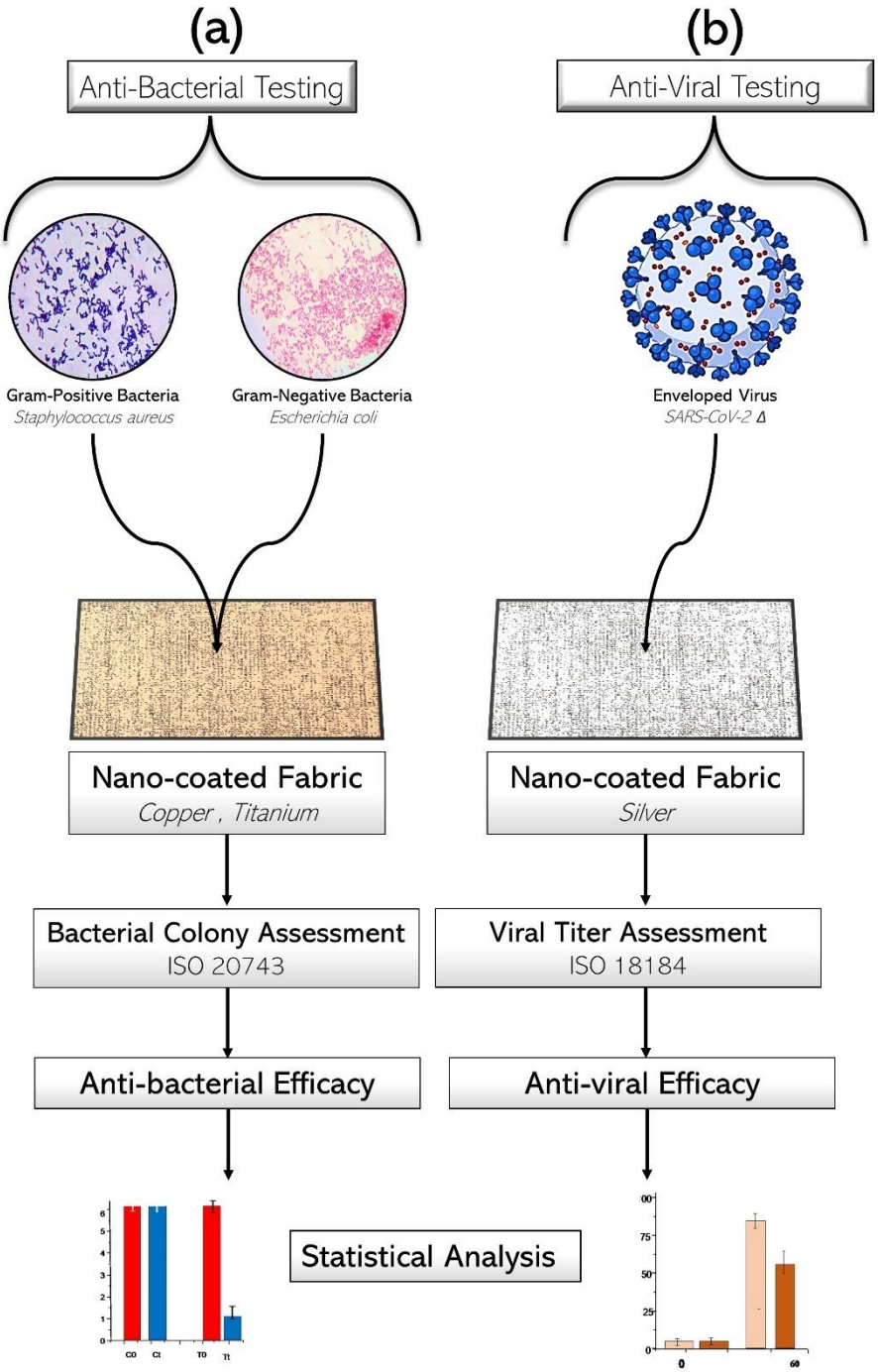
A more advanced method would have been to measure the viral titer via Real-time PCR. But the expense was heavy, and it is more convenient to measure the cytopathic effect via microscope and use the established formula for calculation.

Both the methods used for bacterial and viral testing were ISO certified and were optimized for fabric testing. The bacterial testing had a pre-requisite of maintaining the CFU number prior to any experimentation as a standard bacterial population is needed initially which is left in the log phase overnight (24h) after which it reaches a standard bacterial inoculum. This can also be assured by checking the optical density of the overnight culture, which should be 0.05

according to the ISO protocol. Similarly, for the viral testing a known viral titer (preferably above 10^6 International Units of Virus) had to be maintained which was initially checked by the Real time PCR report of the patient and secondly it was also maintained in the lab using TCID50 protocol. That viral titer was used throughout the testing. If the titer was consumed and a new titer was required, then the virus was propagated in the flask with cells and the DMEM was stored which was quantified using the TCID50 protocol. The study by (Shan, Yang, Wang, & Tian, 2016) showed that the TCID 50 assay is more sensitive than the plaque assay and gave consistent results while three different Real time PCR methods gave non-coherent results. The TCID 50 method is validated as the initial titer of the virus was measured by this technique as well and the average decrease in the viral titer is given in terms of PFUs of the virus.

Chapter 6

Conclusion & Prospects



Promising results were shown by the fabrics tested against both the bacteria and viral microbes which open gateways to further research in this area of E-textiles. A significant decrease in both the bacterial colonies and the viral titer was found after immediate as well as 24hour contact. ISO protocols were used to produce the results which indicate the reproducibility of the methods used. The fabrics were tested after washing as well to assess the strength and sustainability of the E-textile.

In consideration with the assessment of personal protective equipment guidance of healthcare organizations across the globe and the available scientific evidence, shortcomings are still in their protocols. Specially, there is insufficient evidence to suggest that PPE protection standards can be safely lowered when frontline healthcare workers are not working in specific 'higher risk acute care areas' or not performing certain AGPs as advised by PHE. A more precautionary approach is ethically justified given the scientific uncertainty of novel SARS-CoV-2 transmission and pathogenesis. Also in future, a combinational of several nano-fabrics would be more beneficial as compared to only a single type of coating. The best performing fabrics from both anti-bacterial, anti-viral and anti-fungal assessment can be used as a combination for better performance. The techniques through which the nano particles are coated or immobilized on the fabrics could be made better for better durability and performance of the E-textiles.

References

References

Andersen, K. G., Rambaut, A., Lipkin, W. I., Holmes, E. C., & Garry, R. F. (2020). The proximal origin of SARS-CoV-2. *Nature medicine, 26*(4), 450-452.

Anfinrud, P., Stadnytskyi, V., Bax, C. E., & Bax, A. (2020). Visualizing speech-generated oral fluid droplets with laser light scattering. *New England journal of medicine, 382*(21), 2061-2063.

Azhar, E. I., Hui, D. S., Memish, Z. A., Drosten, C., & Zumla, A. (2019). The middle east respiratory syndrome (MERS). *Infectious Disease Clinics, 33*(4), 891-905.

Basodan, R., Park, B., & Chung, H.-J. (2021). Smart Personal Protective Equipment (PPE): Current PPE Needs, Opportunities for Nanotechnology and e-Textiles. *Flexible and Printed Electronics*.

Bastos, C. A., Faria, N., Wills, J., Malmberg, P., Scheers, N., Rees, P., & Powell, J. J. (2020). Copper nanoparticles have negligible direct antibacterial impact. *NanoImpact, 17*, 100192.

Behbudi, G. (2021). Effect of silver nanoparticles disinfectant on covid-19. *Advances in Applied NanoBio-Technologies, 2*(2), 63-67.

Belen-Apak, F., & Sarialioglu, F. (2020). The old but new: Can unfractioned heparin and low molecular weight heparins inhibit proteolytic activation and cellular internalization of SARS-CoV2 by inhibition of host cell proteases? *Medical hypotheses, 142*, 109743.

Beyene, H. D., Werkneh, A. A., Bezabh, H. K., & Ambaye, T. G. (2017). Synthesis paradigm and applications of silver nanoparticles (AgNPs), a review. *Sustainable materials and technologies, 13*, 18-23.

Bhargava, A., Fukushima, E. A., Levine, M., Zhao, W., Tanveer, F., Szpunar, S. M., & Saravolatz, L. (2020). Predictors for severe COVID-19 infection. *Clinical Infectious Diseases, 71*(8), 1962-1968.

Bischoff, W. E., Swett, K., Leng, I., & Peters, T. R. (2013). Exposure to influenza virus aerosols during routine patient care. *The Journal of infectious diseases, 207*(7), 1037-1046.

Bisenius, B. (2017). Product safety of the internet of things [product safety perspectives]. *IEEE Consumer Electronics Magazine, 6*(3), 137-139.

Boopathi, S., Poma, A. B., & Kolandaivel, P. (2021). Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment. *Journal of Biomolecular Structure and Dynamics, 39*(9), 3409-3418.

Bourouiba, L. (2020). Turbulent gas clouds and respiratory pathogen emissions: potential implications for reducing transmission of COVID-19. *Jama, 323*(18), 1837-1838.

Bramstedt, K. A. (2020). COVID-19 as a cause of death for Catholic priests in Italy: an ethical and occupational health crisis. *Health and Social Care Chaplaincy, 8*(2), 180-190.

Chan, J. F., Yuan, S., & Kok, K. (2020). To Kelvin KW, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *The Lancet [revista en internet]*, 514-523.

Chen, H., Guo, J., Wang, C., Luo, F., Yu, X., Zhang, W., . . . Gong, Q. (2020). Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. *The lancet, 395*(10226), 809-815.

Chen, L., Deng, C., Chen, X., Zhang, X., Chen, B., Yu, H., . . . Sun, X. (2020). Ocular manifestations and clinical characteristics of 535 cases of COVID-19 in Wuhan, China: a cross-sectional study. *Acta Ophthalmologica*, *98*(8), e951-e959.

Chustecka, Z. (2020). More than 60 doctors in Italy have died in COVID-19 pandemic. *Medscape.[Accessed on: March 30, 2020]. Available from URL: https://www. medscape. com/viewarticle/927753*.

Cioffi, N., Ditaranto, N., Torsi, L., Picca, R. A., De Giglio, E., Sabbatini, L., . . . Zambonin, P. (2005). Synthesis, analytical characterization and bioactivity of Ag and Cu nanoparticles embedded in poly-vinyl-methyl-ketone films. *Analytical and bioanalytical chemistry*, *382*(8), 1912-1918.

Ciotti, M., Ciccozzi, M., Terrinoni, A., Jiang, W.-C., Wang, C.-B., & Bernardini, S. (2020). The COVID-19 pandemic. *Critical reviews in clinical laboratory sciences*, *57*(6), 365-388.

Colavita, F., Lapa, D., Carletti, F., Lalle, E., Bordi, L., Marsella, P., . . . Corpolongo, A. (2020). SARS-CoV-2 isolation from ocular secretions of a patient with COVID-19 in Italy with prolonged viral RNA detection. *Annals of internal medicine*, *173*(3), 242-243.

Corman, V. M., Muth, D., Niemeyer, D., & Drosten, C. (2018). Hosts and sources of endemic human coronaviruses. *Advances in virus research*, *100*, 163-188.

Dan, Y. Y., Tambyah, P. A., Sim, J., Lim, J., Hsu, L. Y., Chow, W. L., . . . Ho, K. Y. (2009). Cost-effectiveness analysis of hospital infection control response to an epidemic respiratory virus threat. *Emerging infectious diseases*, *15*(12), 1909.

Daniel, J. (2020). Education and the COVID-19 pandemic. *Prospects*, *49*(1), 91-96.

Davidson, C. S., Green, C. F., Gibbs, S. G., Schmid, K. K., Panlilio, A. L., Jensen, P. A., & Scarpino, P. V. (2013). Performance evaluation of selected N95 respirators and surgical masks when challenged with aerosolized endospores and inert particles. *Journal of occupational and environmental hygiene*, *10*(9), 461-467.

De, M., Ghosh, P. S., & Rotello, V. M. (2008). Applications of nanoparticles in biology. *Advanced Materials*, *20*(22), 4225-4241.

Dong, L., Tian, J., He, S., Zhu, C., Wang, J., Liu, C., & Yang, J. (2020). Possible vertical transmission of SARS-CoV-2 from an infected mother to her newborn. *Jama*, *323*(18), 1846-1848.

Draz, M. S., & Shafiee, H. (2018). Applications of gold nanoparticles in virus detection. *Theranostics*, *8*(7), 1985.

Fahmi, I. (2019). World Health Organization coronavirus disease 2019 (Covid-19) situation report. *DroneEmprit*.

Fasogbon, S. A., Nnorom, S. C., Fasogbon, L. O., Adebayo, A. O., Omisakin, I. A., Ogunjimi, T. S., . . . Anya, K. K. COVID-19: THE ROLE OF WELFARE AND SAFETY OF HEALTH WORKERS IN COMBATING THE OUTBREAK.

French, C. E., McKenzie, B. C., Coope, C., Rajanaidu, S., Paranthaman, K., Pebody, R., . . . Beck, C. R. (2016). Risk of nosocomial respiratory syncytial virus infection and effectiveness of control measures to prevent transmission events: a systematic review. *Influenza and Other Respiratory Viruses*, *10*(4), 268-290.

Gao, Y., Yu, L., Yeo, J. C., & Lim, C. T. (2020). Flexible hybrid sensors for health monitoring: materials and mechanisms to render wearability. *Advanced Materials*, *32*(15), 1902133.

Gawn, J., Clayton, M., Makison, C., & Crook, B. (2008). Evaluating the protection afforded by surgical masks against influenza bioaerosols: gross protection of surgical masks compared to filtering facepiece respirators. *Health Safety Exec*.

Giuffrida, A., & Tondo, L. (2020). As if a storm hit': more than 40 Italian health workers have died since crisis began. *The Guardian, 26*.

Greulich, C., Braun, D., Peetsch, A., Diendorf, J., Siebers, B., Epple, M., & Köller, M. (2012). The toxic effect of silver ions and silver nanoparticles towards bacteria and human cells occurs in the same concentration range. *RSC advances, 2*(17), 6981-6987.

Hamming, I., Timens, W., Bulthuis, M., Lely, A., Navis, G. v., & van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 203*(2), 631-637.

Han, Q., Lin, Q., Ni, Z., & You, L. (2020). Uncertainties about the transmission routes of 2019 novel coronavirus. *Influenza and Other Respiratory Viruses, 14*(4), 470.

Henry, B. M., Benoit, S. W., de Oliveira, M. H. S., Hsieh, W. C., Benoit, J., Ballout, R. A., . . . Lippi, G. (2020). Laboratory abnormalities in children with mild and severe coronavirus disease 2019 (COVID-19): a pooled analysis and review. *Clinical biochemistry, 81*, 1-8.

Holland, M., Zaloga, D. J., & Friderici, C. S. (2020). COVID-19 Personal Protective Equipment (PPE) for the emergency physician. *Visual journal of emergency medicine, 19*, 100740.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., . . . Gu, X. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The lancet, 395*(10223), 497-506.

Hui, D. S., Chow, B. K., Chu, L., Ng, S. S., Lee, N., Gin, T., & Chan, M. T. (2012). Exhaled air dispersion during coughing with and without wearing a surgical or N95 mask. *PloS one, 7*(12), e50845.

Hui, D. S., & Zumla, A. (2019). Severe acute respiratory syndrome: historical, epidemiologic, and clinical features. *Infectious Disease Clinics, 33*(4), 869-889.

Jefferson, T., Del Mar, C. B., Dooley, L., Ferroni, E., Al-Ansary, L. A., Bawazeer, G. A., . . . Beller, E. M. (2020). Physical interventions to interrupt or reduce the spread of respiratory viruses. *Cochrane database of systematic reviews*(11).

Katz, A., Alimova, A., Xu, M., Rudolph, E., Shah, M. K., Savage, H. E., . . . Alfano, R. R. (2003). Bacteria size determination by elastic light scattering. *IEEE Journal of Selected Topics in Quantum Electronics, 9*(2), 277-287.

Khan, Y., Ostfeld, A. E., Lochner, C. M., Pierre, A., & Arias, A. C. (2016). Monitoring of vital signs with flexible and wearable medical devices. *Advanced Materials, 28*(22), 4373-4395.

Kośla, K., Olejnik, M., & Olszewska, K. (2020). Preparation and properties of composite materials containing graphene structures and their applicability in personal protective equipment: A Review. *Reviews on Advanced Materials Science, 59*(1), 215-242.

Kumar, D., Malviya, R., & Sharma, P. K. (2020). Corona virus: a review of COVID-19. *EJMO, 4*(1), 8-25.

Lan, Y., Lu, Y., & Ren, Z. (2013). Mini review on photocatalysis of titanium dioxide nanoparticles and their solar applications. *Nano energy, 2*(5), 1031-1045.

Lang, J., Yang, N., Deng, J., Liu, K., Yang, P., Zhang, G., & Jiang, C. (2011). Inhibition of SARS pseudovirus cell entry by lactoferrin binding to heparan sulfate proteoglycans. *PloS one, 6*(8), e23710.

Leung, N. H., Chu, D. K., Shiu, E. Y., Chan, K.-H., McDevitt, J. J., Hau, B. J., . . . Peiris, J. M. (2020). Respiratory virus shedding in exhaled breath and efficacy of face masks. *Nature medicine, 26*(5), 676-680.

Lewis, D. (2020). Is the coronavirus airborne? Experts can't agree. *nature, 580*(7802), 175.

Lindsley, W. G., King, W. P., Thewlis, R. E., Reynolds, J. S., Panday, K., Cao, G., & Szalajda, J. V. (2012). Dispersion and exposure to a cough-generated aerosol in a simulated medical examination room. *Journal of occupational and environmental hygiene, 9*(12), 681-690.

Lindsley, W. G., Noti, J. D., Blachere, F. M., Szalajda, J. V., & Beezhold, D. H. (2014). Efficacy of face shields against cough aerosol droplets from a cough simulator. *Journal of occupational and environmental hygiene, 11*(8), 509-518.

Lippi, G., & Plebani, M. (2020). The critical role of laboratory medicine during coronavirus disease 2019 (COVID-19) and other viral outbreaks. *Clinical Chemistry and Laboratory Medicine (CCLM), 58*(7), 1063-1069.

Lu, C.-w., Liu, X.-f., & Jia, Z.-f. (2020). 2019-nCoV transmission through the ocular surface must not be ignored. *Lancet (London, England), 395*(10224), e39.

MacIntyre, C. R., & Chughtai, A. A. (2015). Facemasks for the prevention of infection in healthcare and community settings. *Bmj, 350*.

MacIntyre, C. R., Mukerji, S., Newall, A., Seale, H., Wang, Q., Wang, X., & Yang, P. (2017). Cost-effectiveness analysis of N95 respirators and medical masks to protect healthcare workers in China from respiratory infections.

Marsh, S. (2020). Doctors, nurses, porters, volunteers: the UK health workers who have died from Covid-19. *The Guardian, 22*.

McFarland, A. D., & Van Duyne, R. P. (2003). Single silver nanoparticles as real-time optical sensors with zeptomole sensitivity. *Nano letters, 3*(8), 1057-1062.

Mohanraj, V., & Chen, Y. (2006). Nanoparticles-a review. *Tropical journal of pharmaceutical research, 5*(1), 561-573.

Nao, N., Yamagishi, J., Miyamoto, H., Igarashi, M., Manzoor, R., Ohnuma, A., . . . Kajihara, M. (2017). Genetic predisposition to acquire a polybasic cleavage site for highly pathogenic avian influenza virus hemagglutinin. *MBio, 8*(1), e02298-02216.

Narayanan, K. B., & Sakthivel, N. (2010). Biological synthesis of metal nanoparticles by microbes. *Advances in colloid and interface science, 156*(1-2), 1-13.

Nicas, M., Nazaroff, W. W., & Hubbard, A. (2005). Toward understanding the risk of secondary airborne infection: emission of respirable pathogens. *Journal of occupational and environmental hygiene, 2*(3), 143-154.

Ohnemus, J., & Rasel, D. (2018). FashionTech-Smart Textiles: Kurzexpertise im Auftrag des BMWi. *ZEW Zentrum für europäische Wirtschaftsforschung: Mannheim, Germany*.

Ong, S. W. X., Tan, Y. K., Chia, P. Y., Lee, T. H., Ng, O. T., Wong, M. S. Y., & Marimuthu, K. (2020). Air, surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a symptomatic patient. *Jama, 323*(16), 1610-1612.

Organization, W. H. (2020a). *Modes of transmission of virus causing COVID-19: implications for IPC precaution recommendations: scientific brief, 29 March 2020*. Retrieved from

Organization, W. H. (2020b). *Rational use of personal protective equipment (PPE) for coronavirus disease (COVID-19): interim guidance, 19 March 2020*. Retrieved from

Pantidos, N., & Horsfall, L. E. (2014). Biological synthesis of metallic nanoparticles by bacteria, fungi and plants. *Journal of Nanomedicine & Nanotechnology, 5*(5), 1.

Paulo, C. S., Vidal, M., & Ferreira, L. S. (2010). Antifungal nanoparticles and surfaces. *Biomacromolecules, 11*(10), 2810-2817.

Rafique, M., Shaikh, A. J., Rasheed, R., Tahir, M. B., Bakhat, H. F., Rafique, M. S., & Rabbani, F. (2017). A review on synthesis, characterization and applications of copper nanoparticles using green method. *Nano, 12*(04), 1750043.

Ray, T. R., Choi, J., Bandodkar, A. J., Krishnan, S., Gutruf, P., Tian, L., . . . Rogers, J. A. (2019). Bio-integrated wearable systems: a comprehensive review. *Chemical reviews, 119*(8), 5461-5533.

Renu, K., Prasanna, P. L., & Gopalakrishnan, A. V. (2020). Coronaviruses pathogenesis, comorbidities and multi-organ damage–A review. *Life Sciences, 255*, 117839.

Reumann, R.-D. (2000). Mechanisches Formänderungsverhalten textiler Materialien. In *Prüfverfahren in der Textil-und Bekleidungstechnik* (pp. 57-125): Springer.

Roberge, R. J. (2016). Face shields for infection control: A review. *Journal of occupational and environmental hygiene, 13*(4), 235-242.

Rotzler, S. (2018). *Einfluss der Sinnerschen Faktoren sowie der textilen Substrate auf die Waschbarkeit textilintegrierter Leiterbahnen.* Hochschule für Technik und Wirtschaft Berlin,

Rotzler, S., Kallmayer, C., Dils, C., von Krshiwoblozki, M., Bauer, U., & Schneider-Ramelow, M. (2020). Improving the washability of smart textiles: influence of different washing conditions on textile integrated conductor tracks. *The Journal of The Textile Institute, 111*(12), 1766-1777.

Rotzler, S., Krshiwoblozki, M. v., & Schneider-Ramelow, M. (2021). Washability of e-textiles: Current testing practices and the need for standardization. *Textile Research Journal*, 0040517521996727.

Ruan, Q., Yang, K., Wang, W., Jiang, L., & Song, J. (2020). Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive care medicine, 46*(5), 846-848.

Sanna, G., Serrau, G., Bassareo, P. P., Neroni, P., Fanos, V., & Marcialis, M. A. (2020). Children's heart and COVID-19: up-to-date evidence in the form of a systematic review. *European journal of pediatrics, 179*, 1079-1087.

Santarpia, J., Rivera, D., Herrera, V., Morwitzer, M., Creager, H., Santarpia, G., . . . Broadhurst, M. Transmission potential of SARS-CoV-2 in viral shedding observed at the University of Nebraska Medical Center. medRxiv 2020: 2020.03. 23.20039446. In.

Shan, L., Yang, D., Wang, D., & Tian, P. (2016). Comparison of cell-based and PCR-based assays as methods for measuring infectivity of Tulane virus. *Journal of virological methods, 231*, 1-7.

Shi, J., Liu, S., Zhang, L., Yang, B., Shu, L., Yang, Y., . . . Chen, W. (2020). Smart textile-integrated microelectronic systems for wearable applications. *Advanced Materials, 32*(5), 1901958.

Smith, J. D., MacDougall, C. C., Johnstone, J., Copes, R. A., Schwartz, B., & Garber, G. E. (2016). Effectiveness of N95 respirators versus surgical masks in protecting health care workers from acute respiratory infection: a systematic review and meta-analysis. *Cmaj, 188*(8), 567-574.

Stoppa, M., & Chiolerio, A. (2014). Wearable electronics and smart textiles: A critical review. *sensors, 14*(7), 11957-11992.

Tao, X. (2001). *Smart fibres, fabrics and clothing: fundamentals and applications*: Elsevier.

Tellier, R., Li, Y., Cowling, B. J., & Tang, J. W. (2019). Recognition of aerosol transmission of infectious agents: a commentary. *BMC infectious diseases, 19*(1), 1-9.

Thakkar, K. N., Mhatre, S. S., & Parikh, R. Y. (2010). Biological synthesis of metallic nanoparticles. *Nanomedicine: nanotechnology, biology and medicine, 6*(2), 257-262.

Thomas, J. P., Srinivasan, A., Wickramarachchi, C. S., Dhesi, P. K., Hung, Y. M., & Kamath, A. V. (2020). Evaluating the national PPE guidance for NHS healthcare workers during the COVID-19 pandemic. *Clinical Medicine, 20*(3), 242.

Tran, K., Cimon, K., Severn, M., Pessoa-Silva, C. L., & Conly, J. (2012). Aerosol generating procedures and risk of transmission of acute respiratory infections to healthcare workers: a systematic review. *PloS one, 7*(4), e35797.

Tricoli, A., Nasiri, N., & De, S. (2017). Wearable and miniaturized sensor technologies for personalized and preventive medicine. *Advanced Functional Materials, 27*(15), 1605271.

Van Doremalen, N., Bushmaker, T., Morris, D. H., Holbrook, M. G., Gamble, A., Williamson, B. N., . . . Gerber, S. I. (2020). Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *New England journal of medicine, 382*(16), 1564-1567.

Wagner, E., Plank, C., Zatloukal, K., Cotten, M., & Birnstiel, M. L. (1992). Influenza virus hemagglutinin HA-2 N-terminal fusogenic peptides augment gene transfer by transferrin-polylysine-DNA complexes: toward a synthetic virus-like gene-transfer vehicle. *Proceedings of the National Academy of Sciences, 89*(17), 7934-7938.

Wang, C., Horby, P. W., Hayden, F. G., & Gao, G. F. (2020). A novel coronavirus outbreak of global health concern. *The lancet, 395*(10223), 470-473.

Wang, C. Z., Hu, S. L., Wang, L., Li, M., & Li, H. T. (2020). Early risk factors of the exacerbation of coronavirus disease 2019 pneumonia. *Journal of Medical Virology, 92*(11), 2593-2599.

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., . . . Xiong, Y. (2020). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. *Jama, 323*(11), 1061-1069.

Wang, W., Xu, Y., Gao, R., Lu, R., Han, K., Wu, G., & Tan, W. (2020). Detection of SARS-CoV-2 in different types of clinical specimens. *Jama, 323*(18), 1843-1844.

Waszczuk, P., Barnard, T. M., Rice, C., Masel, R. I., & Wieckowski, A. (2002). A nanoparticle catalyst with superior activity for electrooxidation of formic acid. *Electrochemistry Communications, 4*(7), 599-603.

Weber, R. T., Phan, L. T., Fritzen-Pedicini, C., & Jones, R. M. (2019). Environmental and personal protective equipment contamination during simulated healthcare activities. *Annals of work exposures and health, 63*(7), 784-796.

Wehr, R. (2017). *Korrelation der Primärstruktur von Xanthanen mit deren Verhalten in Lösung.* Johannes Gutenberg-Universität Mainz,

Wu, F., Zhao, S., Yu, B., Chen, Y.-M., Wang, W., Song, Z.-G., . . . Pei, Y.-Y. (2020). A new coronavirus associated with human respiratory disease in China. *nature, 579*(7798), 265-269.

Yan, J., Grantham, M., Pantelic, J., De Mesquita, P. J. B., Albert, B., Liu, F., . . . Consortium, E. (2018). Infectious virus in exhaled breath of symptomatic seasonal influenza cases from a college community. *Proceedings of the National Academy of Sciences, 115*(5), 1081-1086.

Yetisen, A. K., Qu, H., Manbachi, A., Butt, H., Dokmeci, M. R., Hinestroza, J. P., . . . Yun, S. H. (2016). Nanotechnology in textiles. *ACS nano, 10*(3), 3042-3068.

Yuen, K., Ye, Z., Fung, S., Chan, C., & Jin, D. (2020). SARS-CoV-2 and COVID-19: the most important research questions. Cell Biosci 10: 40. *Link: https://bit. ly/31zLc1Y*.

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., . . . Huang, C.-L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *nature, 579*(7798), 270-273.

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., . . . Lu, R. (2020). A novel coronavirus from patients with pneumonia in China, 2019. *New England journal of medicine*.

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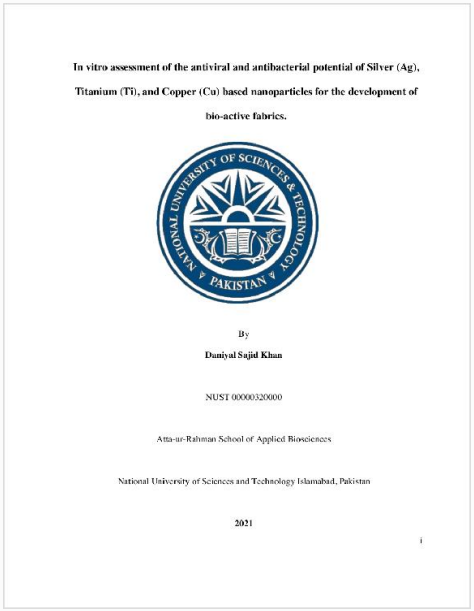

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