

**Evaluation of antimicrobial coatings in wet conditions and
development of sulphonated poly (ether ether ketone) – copper
composites for antimicrobial applications**

by

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Author's declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Statement of contributions

I would like to state that I am the sole author of Chapter 1, 2, 3 and 5.

Chapter 4 is an unpublished manuscript titled *Recyclable antimicrobial sulphonated poly(ether ether ketone) – copper films: flat vs micro-pillared surfaces*, submitted to Materials Today Communication journal, which is co-authored with Lukas Bauman (PhD candidate, University of Waterloo) and reviewed by my supervisor Professor Boxin Zhao and co-supervisor Professor William A. Anderson.

Abstract

The existence of pathogenic bacteria and fungi on surfaces can be a serious threat to health causing numerous infections. With microorganisms developing antimicrobial resistance over the years, there is an increasing need to develop surfaces that can kill or inhibit the growth of bacteria and fungi. Antimicrobial coatings have been tested as an effective solution to battle hospital-acquired infections, which are one of the leading causes for patient morbidity and mortality. Aereus Technologies has been working on developing a coating consisting of marine paint and biocidal copper-alloy based microparticles that can render surfaces antimicrobial. One of the key concerns for this new coating is that the microparticles are expected to oxidize when exposed to human palm sweat or disinfection agents in healthcare settings. To investigate this possibility, the antimicrobial efficacy and durability of this coating was evaluated under exposure to different saline environments (artificial ocean water and artificial sweat environment) and a strongly oxidizing environment (hydrogen peroxide). From the experiments, it was observed that regular painted and marine painted samples with proprietary “Aereus shield” particles have the ability to survive harsh oxidizing conditions without losing their antimicrobial properties.

The antimicrobial application of copper was extended to the synthesis of sulphonated poly(ether ether ketone) (SPEEK) and copper composite films as promising antimicrobial materials. The effects of fabricating surface structures (micro-pillars) on the films were investigated. The synthesized films were systematically characterized and it was observed that both SPEEK and SPEEK – Cu films possessed antimicrobial properties which initially demonstrated over 4 log reduction of *E. coli* within 15 minutes of contact. Over longer times as the films aged, the SPEEK – Cu film still achieved over 2 log (for flat film) and 4 log (for micro-pillared film) reduction of *E. coli* within 1 hour of contact and showed significant fungal growth reduction for *Saccharomyces cerevisiae* (yeast) and *Aspergillus niger* after 2 hours of contact. Micro-pillared films had a larger water contact angle (131°) in comparison to flat films (64°), indicating that they were hydrophobic.

These micro-pillared films also showed better antimicrobial properties and more copper release than flat films, due to better exposure of copper particles on the top surface of the micro-pillars. The synthesized films were also thermally stable up to 300°C and exhibited tensile strengths ranging from 50 – 160 MPa depending on the surface morphology and copper content. Furthermore, the films were found to be recyclable by dissolution in sulphuric acid and re-casting to form new films with replenished sulphonic acid groups in the polymer matrix and restored antimicrobial properties.

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List of Abbreviations

PEEK	Poly (ether ether ketone)
SPEEK	Sulphonated poly (ether ether ketone)
Cu	Copper
NMP	N-Methyl-2-pyrrolidone
PDMS	Polydimethylsiloxane
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
ESEM	Environmental Scanning Electron Microscope
EDS	Energy Dispersive X-ray Spectroscopy
EPA	Environmental Protection Agency
TGA	Thermogravimetric Analysis
DSC	Differential Scanning Calorimetry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry

Chapter 1

Introduction

The simple contact adhesion of pathogenic bacteria results in the formation of bacterial colonies, which then multiply and disperse, causing infections that may be difficult to treat with conventional touch surfaces [1]. Hospital acquired infections (HAIs) are secondary infections initiated during a patient's stay in a healthcare setting and are one of the world's leading causes for morbidity and mortality. In the United States alone, HAIs have been estimated to affect 4-10% of all admitted patients, costing \$5 to \$10 billion in healthcare and greater than 99,000 lives annually [2]–[6]. Statistics in Canada appear to be proportionately similar, with approximately 8,000 deaths reported per year [7].

It is well accepted that bacteria survive by attaching to solid surfaces – forming colonial structures called biofilms that can make bacteria drastically more resistant to antimicrobial surfaces and external forces like cleaning and serve as a reservoir for transmission. While there has not been direct scientific evidence, it is well noted with mounting indirect evidence that high touch surfaces such as keyboards, control panels, bed rails, and handles in the healthcare environment can aid the spread of infections via direct hand-to-surface contact. Since these surfaces are usually made of stainless steel and plastics that do not have inherent antibacterial properties, the current practice is to repeatedly wipe these surfaces with aggressive biocidal solutions to reduce risk of transmission. This practice, in addition to being inefficient (i.e. cumbersome and temporary), can be ineffective. For instance, one study detected methicillin-resistant *Staphylococcus aureus* (i.e. a common pathogen) on 74% and 66% of swab samples in a hospital environment before and after cleaning, respectively [8]. The ability of a microorganism to survive and multiply in the presence of an antimicrobial agent that would inhibit or kill that particular kind of organism is called antimicrobial resistance. This ability is one of the many traits that resilient bacterial subpopulations may possess or acquire, enabling them to overcome host strategies aimed against them. In the face of antibiotic resistance and the emergence of multi-drug resistant superbugs, there is an urgent need for a better solution to combat the transmission of bacteria

to prevent HAIs. Antimicrobial touch surfaces play a crucial role in preventing the growth and transmission of bacteria. Studies also show that microorganisms possess the ability to survive on surfaces for a considerable amount of time [9], [10]. To combat this, novel antimicrobial surfaces are constantly being developed.

With new developments in polymers strengthening their resistance to wear and copper being a comparatively cheap material possessing antimicrobial properties, copper-polymer composites are being explored for the development of antimicrobial touch surfaces. However, existing copper-polymer surfaces are mostly non-recyclable, have issues with exposure of copper particles on the surface or their antimicrobial properties are limited as they decrease with aging [11][12]. Poly (ether ether ketone) (PEEK) is a high temperature, high performance thermoplastic that displays desirable mechanical properties and resistance to chemicals, abrasion, hydrolysis and many other properties. Additionally, it has a low density along with self-lubricating properties and good flowability, allowing ease of processing. Furthermore, using appropriate fillers can further improve its lubricating properties and mechanical strength. However, PEEK by itself does not have any reported inherent antimicrobial properties; studies always heavily rely on the modification of PEEK surfaces by either sulphonation or addition of fillers to render the surface antimicrobial [13]–[17].

Significant research has investigated the sulphonation of PEEK by sulphuric acid. The resulting SPEEK can then be solution cast using N-Methyl-2-Pyrrolidone (NMP) to form SPEEK films, which exhibit excellent antimicrobial properties [18]. While the underlying biocidal mechanism is still under exploration, it is generally understood that on contact, water on the surface of the microbes interacts with the sulphonic acid functional groups in the polymer, creating an acidic solution that quickly kills the bacteria [19]. The addition of copper microparticles into the SPEEK matrix can further enhance its antibacterial/antifungal properties. The antimicrobial action of copper generally involves the release of metallic ions, which can directly rupture microbial cell walls and degrade the microbial proteins and DNAs. It can also indirectly damage the microbes via the generation of reactive oxygen species. The associated biocidal activity is especially high when the microbes are in direct contact with the inorganic materials. For instance, it was found that under dry direct contact, metallic copper can eliminate and

inactivate microbes at a rate of at least 7–8 logs per hour (i.e. effective killing in minutes) [20]. Log reduction is a measurement which quantitatively describes how effective an antimicrobial surface reduces the concentration of a microorganism. Mathematically, it is a logarithmic ratio of initial number of viable organisms to number of viable organisms after treatment or contact with a surface for the given time period, so, more the log reduction, more is effective the killing in minutes (1 log = 90% reduction, 2 log = 99.99% reduction, 4 log = 99.99% reduction and so on). Therefore, this is orders of magnitude higher than without direct contact; furthermore, no known microbe can survive or develop resistance after prolonged incubation. This “contact killing” phenomenon, which has been confirmed to be both prominent and long-lasting in laboratory clinical environments, is highly desirable for high-touch surfaces [21]. Yet, the adaptation of these materials out of the lab has not been very successful so far due to prohibitively high costs and manufacturing challenges. To resolve this issue, numerous studies have been devoted to making high-touch surfaces biocidal through either surface bonding of antimicrobial inorganic nanoparticles [22]–[27] (e.g. ZnO, Ag and CuO) or coating the surfaces with polymers containing the nanoparticles [28]–[37]. Neither approach is ideal. The adhesion between the surface and the particles is a concern for the former, while potentially poor biocidal efficacy is a concern for the latter due to covering of the particles with polymer [38], [39]. For further detail on this subject, a literature review is presented in Chapter 2 which briefly reviews the background of antimicrobial polymer coatings.

Chapter 3 presents a prequel to the main objective of this project, wherein the antimicrobial efficacy of samples coated with a copper-containing paint were tested after immersion in saline and under rapid oxidizing conditions as part of an industrial project (Aereus Technologies Inc.). This side-project was product development work involving characterization of antimicrobial touch surfaces that the company manufactured. The work presented in Chapter 3 was an exploration of how environmental factors might affect the longer-term performance of a copper-enhanced antimicrobial surface.

Herein, we report the synthesis of sulphonated poly (ether ether ketone) (SPEEK) – copper films and investigate the possibility of their use as antimicrobial materials in Chapter 4. A novel method has been applied in this work which involves solution casting SPEEK films in polydimethylsiloxane

(PDMS) negative micro-pillar molds. This method produces films with micro-pillars on the surface, exposing the copper micro-particles to a greater extent on the surface. The differences in the level of copper micro-particle exposure, other physical and chemical properties and antimicrobial properties are all systematically studied, making a direct comparison with flat films. In Chapter 5, concluding remarks are presented to summarize the determinations of this thesis and provide recommendations for future work.

Chapter 2

Literature Review

The adhesion and growth of microbes on the surface of most biomedical materials can cause serious health problems and are regarded as a fundamental cause of infections. Antimicrobial resistance has been an increasingly important concern for using antimicrobial agents, which inhibits or kills the microorganism but renders the microorganism to develop resistance. It is shocking to note the rate in which these microorganisms develop antimicrobial resistance and how quickly it spreads across the globe and among different species of bacteria [40], [41]. Due to this rapidity, there is a constant need to develop new and better antimicrobial coatings that would inhibit or kill these microorganisms. There are numerous principles that have been investigated for synthesizing antimicrobial coatings. The main principles that will be focused on in this work are preventing adhesion of bacteria and killing microorganisms either before or after contact is made with surface. This literature review will discuss some of the antimicrobial polymer coatings following these principles that have been considered as an effective mitigation strategy of bacterial pathogens.

2.1 Antiadhesive coatings

Surfaces that are both superhydrophilic and neutral can provide many advantages for antiadhesive purposes; *in vitro* studies corroborate that they inhibit several microorganism species from biomaterial adhesion by limiting the contact between bacteria and potential surface placement sites of materials. To this end, Rojo et al. employed bulk polymerization reaction to prepare high conversion copolymers from the hydrophilic monomer 2-hydroxyethyl methacrylate (HEMA) and different eugenol monomeric derivatives, eugenyl methacrylate (EgMA) and ethoxyeugenyl methacrylate (EEgMA) which acted as an effective antiadhesive coating [42]. As an alternative to fabricating bulk polymers, work by Campoccia et al. suggests that treating macromolecule surfaces and/or modifying interactions between protein-bacteria is also a decent strategy for reducing microorganism adhesion to a selected biomaterial [43]. It was also observed by Wilson et al. that proteins like fibronectin,

fibrinogen, laminin, denaturated collagens, and a few plasma/tissue lipids are the primary host substances that move with the surface structure of the biomaterial, suggesting their adhesion is what needs to be minimized [44]. Studies by Yeo et al. indicated that diminution of conditional lipid-protein layer formation may be accomplished by dynamic surface chemistry characteristics (like roughness, free energy) and/or surface micromorphology [45]. In fact, a variety of studies by Bacakova et al. and Hauslich et al. have corroborated that the biological response to biomaterials may be controlled via alterations in surface chemistry and structure [46], [47]. As an example, it has been proposed that greater bacterial adhesion will tend to occur on surfaces that are porous and rough in comparison to smooth surfaces, which could be because of a larger real contact area for microorganism adhesion and an ulterior higher variety of anchor points [48]. Supporting this, some studies report that the usage of porous materials is related to enlarged risk of infection as compared with cemented ones. As such, the formation of a thick macromolecule layer on the surface of materials with high roughness may suppress bacterium adhesion. As well as roughness, surface chemistry and structure may play an important role; the adhesion method may be completely different among materials with different surface structures in terms of short-range van der Waals interactions and surface energy, as illustrated in Figure 2.1 [49]–[52].

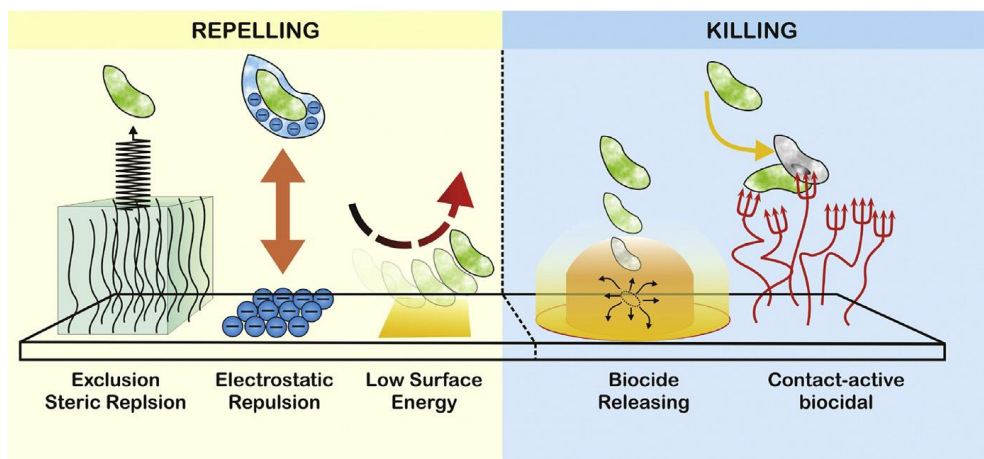


Figure 2. 1: Strategies for creating antimicrobial surface [1] Reproduced with permission from Elsevier

2.2 Nanostructured surfaces and coatings

Research into nanostructured surfaces and coatings is presently being pursued with great interest by the scientific community, as they have a minimal number of biocompatibility issues. As a result, nanoscale surface patterning techniques have been applied to fabricate a large variety of nanopatterns. Many studies corroborate that nanopatterning together with different surface treatments might inhibit microorganism adhesion [53]. Another application is the fabrication of polymers containing bactericidal nanoparticles and substances that inhibit dormant and sessile microorganisms. Derivatives of both synthetic and natural polymers has been well researched for antibacterial purposes. For example, Lakshmi S. Nair et al. talk about silver nanoparticles for wound-healing applications and nanostructures for orthopaedic applications like a) nanophase materials composed of alumina, titania and hydroxyapatite with grain size < 100 nm, which have demonstrated enhanced osteoblast and osteoclast activity; b) use of biodegradable polymers like polyphosphazenes and poly(DL-lactic-co-glycolic acid) to fabricate polymeric nanofibers for enhancing osteoblast adhesion and proliferation [54].

One bactericidal agent which shows promise is zinc oxide, which has shown a higher bactericidal activity than any other metal oxide nanoparticle. It was also found that zinc oxide not only kills bacteria that are subjected to UV light but also inhibits the growth of microorganisms under visible light [55]. When zinc oxide is exposed to ultraviolet light with wavelengths less than 388 nm, can generate valence band holes and conduction band electrons which subsequently produce radicals that can destroy and shrink bacterial cells by reacting with the organism in a photocatalytic process [56]. As summarized in references, the advantages of this method include excellent photocatalytic and antimicrobial activity, nontoxicity and biocompatibility, strong physicochemical stability, and durable antibacterial properties. The antibacterial mechanism of semiconductor photocatalysis is mainly based on this ultraviolet photoactive property.

To increase practical usage of bactericidal agents, several studies have been done to coat zinc oxide films on the surface of several materials that see wide everyday use, like glass, ceramic, chrome

steel, polymer and so on. In practice, to achieve extremely adherent zinc oxide film with a high bactericidal activity, high temperature heating of the materials to be coated is needed. Since most polymers have a low heat resistance, zinc oxide films cannot coat them well, which is the main barrier to their practical use.

2.3 Surfaces with antibacterial properties

The two main methods that are advised for effective medical surface treatment involve either contact killing or drug elution. Figure 2.2 illustrates the contact killing approach. Surfaces that kill microorganisms depend on various mechanisms of action, which can interfere with a cell's respiration, organic process, or formation of a cytomembrane. Another promising approach involves interfering with the microorganism's signaling network or impeding its transition into a sessile development of microbial cells (i.e. biofilm). The research of Chagnot et al. says that bacterial adhesion paves the way for biofilm formation, but not all single bacterial cells that adhere reversibly or irreversibly engage inexorably into a sessile mode of growth. Their research also says that surface proteins promote bacterial colonization and that a protein must be secreted to be present on the cell surface of the bacteria [57]. This maneuver may prolong the window of chance for prophylactic antimicrobial activity and therefore the host immunologic response.

There are many antibacterial surface technologies which use metals (silver, copper, etc.), nonmetals (such as selenium), organic substances (antibiotics, chitosan, alternative substances) and their combinations. For most of metal coatings, their antibacterial activity is closely linked to being in an ionic or nanoparticle form, rather than the bulk material [58]. In spite of extensive research and studies in this field, thin layer metal coatings are still not standard antimicrobials for medical implants [59]. This is because of their cytotoxicity and the resulting decreased biocompatibility, which prevents their widespread use. Additionally, it still remains a tough task to create a tough interface to sustain the mechanical stresses involved in surgical implant insertion and ultimate loading once *in vivo* [60]. Furthermore, the publication of Darouiche et al. about antimicrobial efficacy of silver-coated heart walls still remains a controversy, as it did not prove that silver-coated sewing rings could be clinically anti-

infective [61]. Because of these issues, in this review we will focus on the role of quaternary ammonium compounds and antimicrobial metals like silver and copper in antimicrobial coatings.

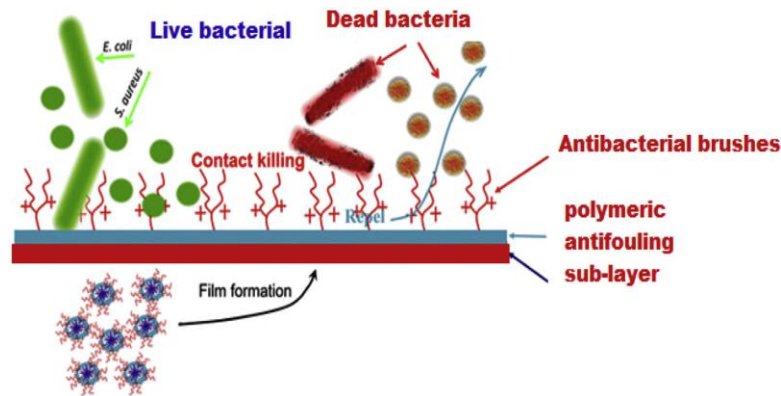


Figure 2. 2: Contact killing approach [1] Reproduced with permission from Elsevier

2.3.1 Quaternary Ammonium Compounds (QACs)

Positively charged surfaces show negative impact on cell survival. Therefore, numerous antimicrobial surfaces and coatings containing quaternary ammonium ions have been developed. Quaternary ammonium compounds are widely utilized as bactericidal agents and the resulting coatings have been found to have a contact-based bactericide mechanism that lasts for a significant amount of time, unlike silver ions that show a release-based bactericidal mechanism. For instance, Radhesh Kumar et al. studied the release of silver ions from a silver-filled polyamide composite system. The change in silver ion release was observed with varying time and concentration of silver powder and was found to be influenced by the specific surface area of the silver powder. As concluding remarks of their publication, the composite system was found to release silver ions at concentration levels capable of providing sufficient antimicrobial efficacy [62]. Regarding quaternary ammonium compounds, some authors like Joerg C. Tiller et al. corroborate that surfaces containing ammonium ion salts or quaternary ammonium ion teams exhibit a harmful impact to gram-positive and gram-negative bacteria cells through disruption of their cell walls [63]. Soluble quaternary ammonium compounds are utilized in industrial applications and water treatment, as well as in pharmaceuticals and daily consumer products.

These quaternary ammonium compounds are usually utilized as protective agents in cosmetic products. Despite this variety of properties, studies have reported resistance of bound microorganisms against quaternary ammonium compound containing surfaces, as well as their cytological and biocompatibility issues (Figure 2.3) [64], [65].

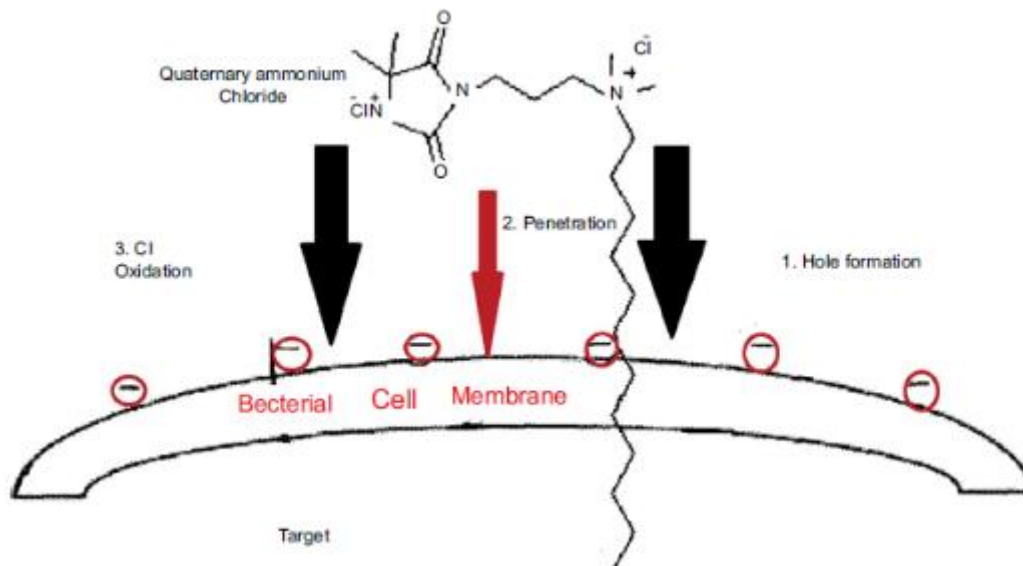


Figure 2. 3: Mechanism of action of quaternary ammonium compounds [1] Reproduced with permission from Elsevier

2.3.2 Surface coating with antimicrobial metals

There are two steps that are involved in the adhesion of bacteria on the surface of metal: first is a primary, quick and reversible physical stage followed by an irreversible molecular and cellular stage that is time-dependent. Forces such as gravitational forces, hydrophobic interactions, van der Waals attraction, surface electrostatic charge effect and Brownian motion play an important role in the movement of bacteria towards the material surface in the first stage. In the second stage, irreversible formation of pili, fimbriae, capsules etc, occur that bridge the bacteria to the material surface. These structural formations happen as a result of molecular reactions between bacterial surface structures and substrate surfaces. Properties such as material chemical compositions, hydrophobicity, surface roughness and charge also influence adhesion of bacteria to the surface [66].

The way metal coatings kill or inhibit bacterial growth usually involves the release of metal ions which rupture the microbial cell wall and damage its proteins and DNA. Since time immemorial, silver and copper have been the two metals which have been extensively used in biomedical applications [67], [68], [11]. This section will discuss their mechanisms of action and list some already existing antimicrobial surfaces that have been proven effective.

2.3.2.1 Silver

When silver is used as an antibacterial agent, it is usually either coated on surfaces in its elemental form, applied within polymer coatings to control silver ion release or impregnated into a polymer matrix in particulate/complex/chelate/salt form [68]–[71]. Silver has been found to be very effective against species like *Escherichia coli*, *Staphylococcus epidermis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. A study of silver's killing mechanism by Georg Gosheger et al. suggested that the microbial cell wall is ruptured and its proteins and DNA are damaged by silver ions [72]. While dissolved silver cations are the predominant agents that are responsible for microbial killing, silver also can form reactive oxygen species (ROS), which is a mechanism that possibly influences prokaryotic cells [73]. However, it has been observed over various studies that even small concentrations of silver can have adverse cytotoxic effects for surrounding cells, also possibly leading to harmful accumulation in distant locations within the cell which is illustrated as in Figure 2.4 [66], [74], [75].

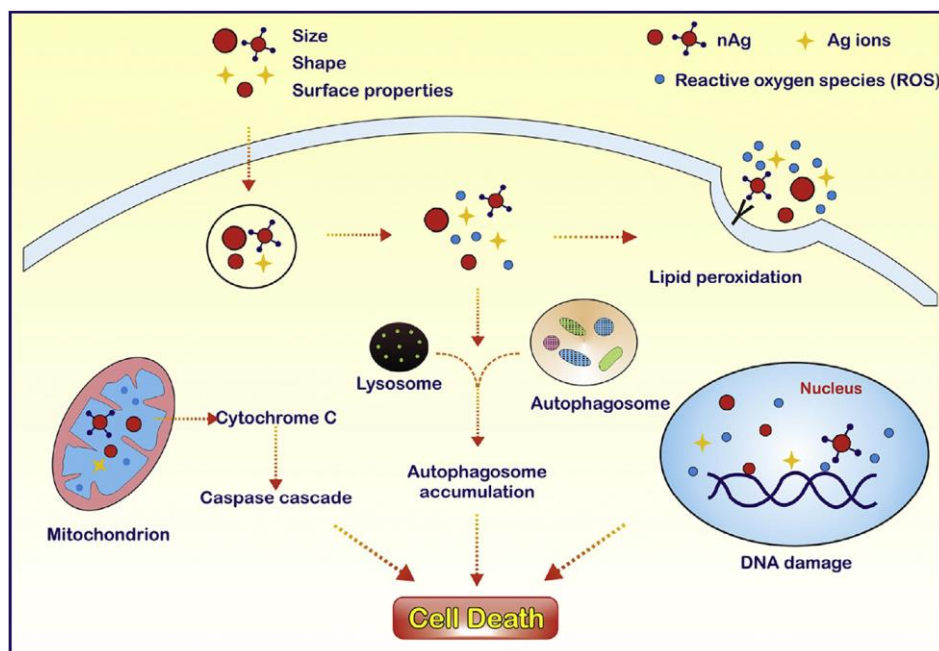


Figure 2. 4: Various modes of action of silver ions against microbes [1] Reproduced with permission from Elsevier

2.3.2.2 Copper

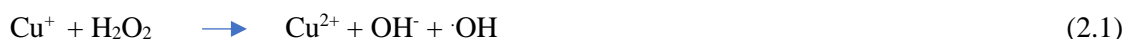
Copper is widely recognized as an antimicrobial agent and it is extensively used in various applications, as its cost is comparatively cheaper than silver. Its rapid bacterial killing ability has received widespread attention. In recent times, copper and copper-based alloys have been approved for use as touch surfaces in hospitals and health-care facilities [76], [77]. Door knobs and other touch surfaces are being made out of copper-based alloys, which exhibit an excellent antimicrobial activity against *E. coli*, methicillin-resistant *S. aureus* (MRSA) and *Clostridium difficile*, influenza A virus, adenovirus and fungi in comparison with regular stainless steel surfaces [78]–[81].

Compared with silver, the antibacterial effects of copper are far less understood; however, scientists accept that the potential antimicrobial mechanisms for copper are the following [11]:

- Oxidative stress caused inside the cell due to elevated copper levels and the generation of hydrogen peroxide lead to a Fenton-type chemical reaction causing oxidative damage to the cells, as illustrated in Figure 2.5

- Cell death occurs as a result of desiccation happening in the cell, due to leakage of specific essential nutrients such as potassium and glutamate caused by excess copper, which compromises the membrane integrity of microbes
- Although certain protein functions require copper, when in excess, it tends to tether itself with proteins that do not require copper for their function. This unnecessary tethering leads to loss of function and thus the breakdown of the protein into non-functional portions

Degradation of the cell membrane is one of the main core functions copper and its compounds perform to kill/inhibit bacterial growth [79]. It was observed from studies of both moist and dry metallic copper surfaces that accumulation of copper ions occurred inside *Staphylococcus haemolyticus*. It was also observed that the absorption of copper kills the bacteria only as a result of the cell membrane being fatally compromised and not by damaging the DNA. In fact, no lethal DNA damage was observed. This concept was further explored by Lemire et al. [80] and Macomber et al. [81], who corroborated that the formation of reactive oxygen species is accompanied by the formation of Cu^{2+} ions, which reduce the capacity of the cell wall to absorb damage from these species and self-repair by accelerating the consumption of antioxidants. The Fenton reaction in Eq. (2.1) describes how the reactive species are generated.



Whenever cellular respiration takes place, hydrogen peroxide is produced as a transient metabolic by-product which as a result leads to this mechanism taking place [80], [82]. However, in the aforementioned study, cell wall damage predominantly occurs due to photocatalytic action, which happens as a result of dissolved copper in titanium dioxide, which is a strongly oxidizing environment. The antimicrobial activity mechanism due to photosensitization of CuO and $\text{ZnO/Fe}_3\text{O}_4$ is illustrated in Figure 2.5.

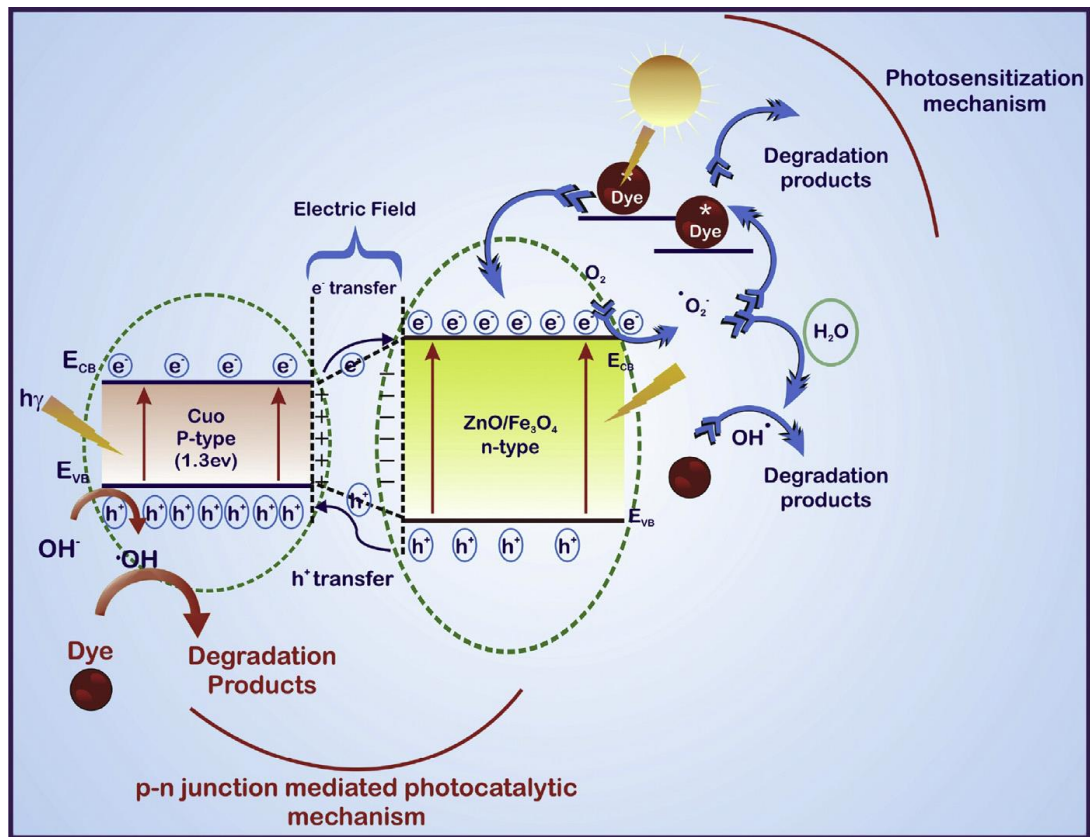


Figure 2. 5: Antimicrobial properties of copper and its alloy by oxidative mechanism [1] Reproduced with permission from Elsevier

Another important aspect of copper particle usage in polymer copper composite is its size. Literature suggests that smaller the size of the particles, more is the particle distribution and release thus corroborating to better antimicrobial action [12]. There is already extensive research presented with nanoparticles [11] and with the larger particle size corresponding less surface area coverage and particle distribution, an optimum size of copper micro-particles will depend on the particular system.

2.4 Antimicrobial polymers and composites

From the above sections, it can be observed that traditionally, antimicrobial activity can correspond to either the presence of groups of covalent linkages in the polymer matrix or the addition of antimicrobial metals within them. The synthesis of polymers that are themselves antimicrobial began as early as 1965 by a group led by Donaruma et al. whose research predominantly focused on polymers and copolymers based on 2-methacryloxytropones, N-acylsulphanilamide, sulphonamide-

dimethylolurea copolymers and sulphonamide or sulphapyridine-formaldehyde copolymers [83]–[88]. The main goal of this research at the time was making water emulsions by blending these polymers with commodity polymers, which were then used as components of protective coatings.

Ever since the discovery of antimicrobial polymers, a lot more polymers have been tapped into for the same application and many reviews have been written to summarize the advancements in this field [89]–[96]. The predominant modes of action have been explained in detail in the previous subsections in this chapter. From all these reviews, for the convenience of this thesis, the antimicrobial polymers can briefly be classified into four types based on their mode of action [94], [97].

- ***Polymers with inherent antimicrobial properties***

Such polymers (as the name suggests) have inherent antimicrobial properties arising from their chemical structure. For example, polymers containing quaternary nitrogen atoms like polysiloxanes, methacrylic polymers, polyionenes etc.; some halogen polymers and polymers mimicking natural peptides like phenylene ethynylene backbone polymers work effectively as antimicrobial agents by disrupting the bacterial cell wall [98]–[105].

- ***Polymers that are chemically modified to become antimicrobial***

When a polymer lacks inherent antimicrobial property, some of them can be chemically modified in such a way that the added functional groups impart antimicrobial activity to the polymer matrix. There are various ways by which this modification can be achieved, the most common of which are: fixing antimicrobial peptides on an inactive polymer, covalently attaching a small molecule with antimicrobial activity to the polymer and grafting antimicrobial polymers to regular polymers. It should be kept in mind that the chemical modification should be done in such a way that it does not majorly deteriorate the properties of the end product [106]–[110].

- **Polymers incorporating antimicrobial organic compounds**

In such a scenario, the antimicrobial activity is imparted by two major sources. One is the blending or mixing of nonactive polymers with antimicrobial polymers to confer their biocidal characteristics, and the other is the establishment of noncovalent links between antimicrobial agents and polymers with corresponding compound release [111]–[114].

- **Polymers with antimicrobial inorganic compounds**

As with the two immediately prior cases, here the polymer has no inherent antimicrobial properties, which are instead obtained by incorporating metals or metal oxides into the polymers to act as the primary source of biocidal action [115]–[119].

Recently, there have been numerous research articles related to the development of copper-polymer composites [11], [120], [121]. The most commonly used polymer substrates are natural polymers, such as cellulose and chitosan and polyolefins, such as polyethylene and polypropylene. The results of some of these novel discoveries have been tabulated in Table 2.1.

Table 2. 1: Different copper-polymer composites and their antimicrobial effects [11] Reproduced with permission from Elsevier

Polymer matrix	Copper nanoparticle size	Microorganism	Innoculus (CFU/mL)	Incubation time/contact time (h)	Antibacterial assay	Effect	Refs
High density polyethylene	200-400	<i>E. coli</i> DHS α , <i>P. fluorescens</i> <i>BS3</i> , <i>S. aureus</i>	1.2×10^5	24	Count of colony-forming units	Significant antibacterial effect for nanocomposites containing 2.5-5 wt%	[122]
Nylon	85	<i>S. aureus</i>	N.A.	16	ATCC 147 method	Inhibition zones	[123]
Polyamine	3-15	<i>C. albicans</i>	10^8	48	Agar diffusion test	Inhibition zone of 12mm against <i>c. albicans</i> at total concentration of copper of 600 mg/100 L	[124]
Polyaniline	50	<i>S. aureus</i> , <i>Bacillus</i> , <i>E. coli</i> , <i>Pseudomonas</i>	10^5	2-10	Agar diffusion test, viability assay, wet interfacial contact method	Disruption of bacterial cell membrane	[125]
Polylactic acid	36	<i>P. fluorescens</i> , <i>P. putida</i>	10^3	24	JIS Z 2801 method	6-log reduction of bacterial	[126]

						growth after 24 h of incubation	
Polypropylene	5	<i>E. coli</i>	10 ⁶	0.5-6	Colony forming unit count	After 4 h, the nanocomposite killed > 99.9% of bacteria	[121]
Polyvinyl alcohol	9.2	<i>E. coli</i> DHSa	10 ⁸	48	Count of colony forming unit	Nanocomposite with 0.6 wt% copper nanoparticles showed a reduction of up to 5-log	[127]
Poly-vinyl-methyl-ketone	1.7-6.3	<i>S. cerevisiae</i> , <i>E. coli</i>	N.A.	48	Colony forming unit count	Significant decrease in the cellular growth with 21 and 0 cfu for <i>E. coli</i> and <i>S. cerevisiae</i> , respectively	[128]

To improve on the above-tabulated research articles, a high-performance thermoplastic, poly (ether ether ketone) (PEEK) was used in this research. As observed from Table 2.2 (which summarizes the properties of a variety of polymers), PEEK was selected for this project because of its exceptional properties. However, since PEEK doesn't possess inherent antimicrobial properties, it was sulphonated to obtain sulphonated poly (ether ether ketone) to which copper micro-particles were then incorporated to produce a novel film with exceptional antimicrobial properties.

Table 2. 2: Properties of PEEK vs other polymers and stainless steel [129]

	Temperature Performance	Chemical Resistance	Dimension Stability	Gamma Radiation	Flex Fatigue	Abrasion Resistance	Relative Tensile Strength	Specific Weight	Costs
PEEK	1	1	1	1	2	2	2	2	4
PTFE	1	1	4	4	1	2	4	4	4
ETFE / E-CTFE	2	2	3	3	2	2	3	3	3
PVDF	2	3	3	3	2	2	3	3	3
PPS	1	2	2	2	3	3	2	2	3
PEN	2	3	2	2	3	3	2	2	1
PET	3	3	2	2	2	3	2	2	1
PA	3	3	3	2	1	1	2	2	2
PP	4	2	3	3	3	4	3	1	1
STAINLESS STEEL	1	2	1	1	4	1	1	4	3

1 = Excellent
2 = Good
3 = Acceptable
4 = Poor

It should also be noted that the above-mentioned properties of PEEK are bound to change to a certain extent when it is sulphonated. But nonetheless, the properties of SPEEK, as discussed in Chapter 4, suggest that its physical and thermal properties are fairly sufficient and above average for its functionality thus making it a promising antimicrobial polymer composite film.

Chapter 3

Evaluating the antimicrobial efficacy of Aereus samples post immersion in gradual and rapid oxidizing environment

3.1 Introduction

Adhesion between a surface coating and particles is a concern for surfaces with antimicrobial inorganic nanoparticles and the biocidal efficacy is a concern for surfaces with polymers containing nanoparticles due to covering of the particles [38], [39]. More importantly, both approaches require the use of specialized processes such as plasma activation [130]–[132] and plasma polymerization [34]–[37], [133], [134] for the coating process, making them difficult to implement with the existing hospital installs. Aereus Technologies, an Ontario start-up company, has recently developed an original antimicrobial coating for touch surfaces that can potentially overcome the above shortcomings.

This approach involves first painting touch surfaces with a wet marine paint and then air-spraying the paint with copper-alloy based microparticles (that are produced by Aereus using a proprietary thermal-spraying process), for which the particles are exposed and are locked in place upon hardening/curing of the paint. We believe the resulting coating would be able to resolve the dilemma between adhesion and efficacy encountered by the previous studies because the microparticles, in addition to being highly biocidal, are morphologically irregular and rough according to our previous work with Aereus, enabling the particles to bond strongly to the paint by mechanical locking while being partly exposed and not fully embedded in the paint. For this coating to be considered as a viable antimicrobial touch surface, it is critical to evaluate its long-term efficacy and durability.

Recent studies suggest that the formation of copper oxides upon oxidation also contribute to the antimicrobial activity of the copper-containing surface, thus shedding some light that oxidation probably has little to no effect on the antimicrobial efficiency of the surfaces [135], [136]. So, to evaluate the long-term efficacy and durability of the coatings produced by Aereus Technologies Inc., in this chapter, we will evaluate the antimicrobial efficacy and durability of this coating using a gradual

oxidizing simulated dynamic saline environment (artificial ocean water and artificial sweat environment) and rapid oxidizing environment (hydrogen peroxide).

3.2 Materials and methods

3.2.1 Experimental setup:

A fish tank as in Figure 3.1 was used for the purpose of simulating an artificial ocean water environment. A pump and a pre-set heater were installed to keep the water in constant and the water temperature at 25°C. The composition of the prepared artificial ocean water and sweat are tabulated in Table 3.1 and 3.2.



Figure 3. 1: The fish tank setup

Table 3. 1: The chemical composition of the artificial ocean water [137]

Reagent	Quantity (for 1 litre)
Sodium chloride	26.29 g
Potassium chloride	0.74 g

Calcium chloride	0.99 g
Magnesium chloride	6.09 g
Magnesium sulphate	3.94 g

Table 3. 2: The chemical composition of the artificial sweat [138]

Reagent	% w/v	Quantity (for 30 litres)
Sodium chloride	1.08	324 g
Lactic acid	0.12	36 g (29.85 mL)
Urea	0.13	39 g

5 different kinds of samples of (1”x1” dimension) provided by Aereus Technologies Inc. were as follows:

1. Non painted
2. Regular painted
3. Regular paint + Aereus shield particles
4. Marine painted
5. Marine paint + Aereus shield particles

The provided samples were then immersed into the fish tank and were taken out periodically (after 5, 8, 12, 15 and 20 days), and tested for antimicrobial efficacy. A Day 0 test was also performed to check the antimicrobial efficacy of the samples prior to immersion in the fish tank. All the experiments were conducted twice and two sets of results were collected which were then averaged.

3.2.2 Protocol for checking the antimicrobial efficacy (EPA protocol) [139]

- The samples were soaked in detergent solution (e.g. Liquinox) for 2-4 hrs to degrease, then rinsed thoroughly with deionized water. and was wiped dry (using kimwipes) and allowed to dry.

- Then the samples were soaked in 95-98% ethanol for 5 to 10 minutes to decontaminate. Sterile forceps were used to remove the samples and it was placed in or on a sterile petri dish in the biosafety cabinet to dry.
- A test culture of *Pseudomonas aeruginosa* in deionized water was prepared. The target level on the stainless-steel control after 60 minutes is 5×10^5 to 5×10^6 CFU/mL, so an applied load of approximately 10^8 CFU/mL is likely satisfactory.
- For each sample, 20 μ L of culture was applied and spread with the sterile pipette tip or sterile loop/spreader over an area of approximately 1 x 1 inch.
- At the end of 120 minutes, the test surfaces were rinsed with 20 mL of sterile de-ionized water captured in the beaker. The contents in the beaker along with the samples were sonicated (60Hz) for 5 minutes to shake of the bacteria from the test surfaces to the de-ionized water.
- After sonication, 5 μ L and 1 μ L of the contents from the beaker were plated in separate PCA plates (plate count agar, *which was prepared by adding 23.5 g of PCA to a 2 litres conical flask and one litre of de-ionized water. The contents were mixed, autoclaved at 121°C for an hour after which they were transferred into petri dishes and cooled to room temperature*) and incubated at about 36°C for 24 hours. The results were then counted and recorded.

3.3 Results

3.3.1 Phosphate buffered saline (PBS) vs de-ionized water

It can be observed that the antimicrobial efficacy tests would have been performed with de-ionized water rather than the conventional PBS that is used. The reason why de-ionized water was used is because to minimize the effect of any interfering ions from the solution that could potentially affect the antimicrobial efficacy of the surfaces. Antimicrobial tests using *E. coli* were performed with a glass control, Nickel, Zinc and Copper with both PBS and de-ionized water as dilution mediums and the results were recorded as in Figure 3.2.

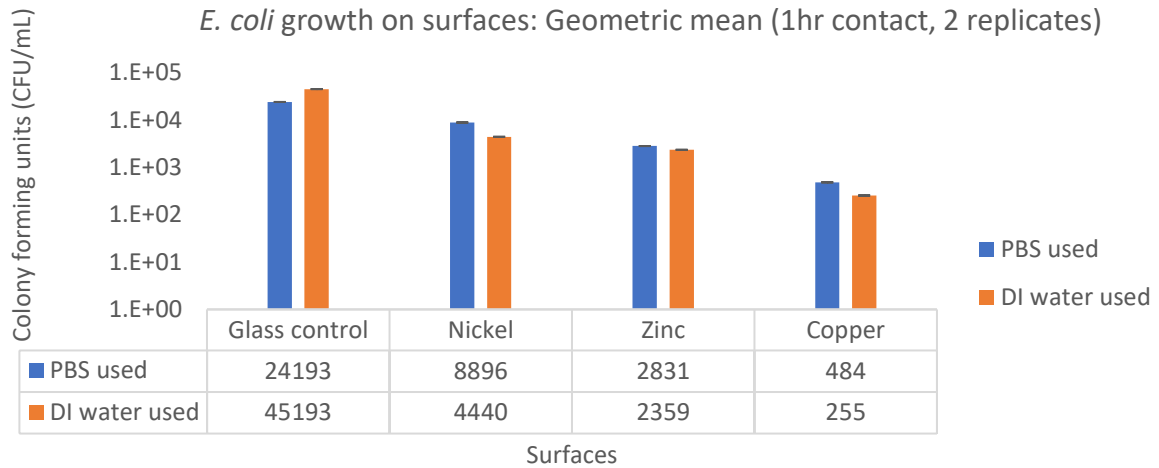


Figure 3. 2: *E. coli* growth on metal surfaces (PBS vs de-ionized water)

It can be observed from Figure 3.2 that there existed a small difference in the collected results when different dilution medium was used. The amount of bacterial growth varied when the dilution medium was changed but the trend was more or less the same. So, for the entirety of this research, de-ionized water was used as the dilution medium for antimicrobial efficacy tests.

3.3.2 Artificial ocean water experiment

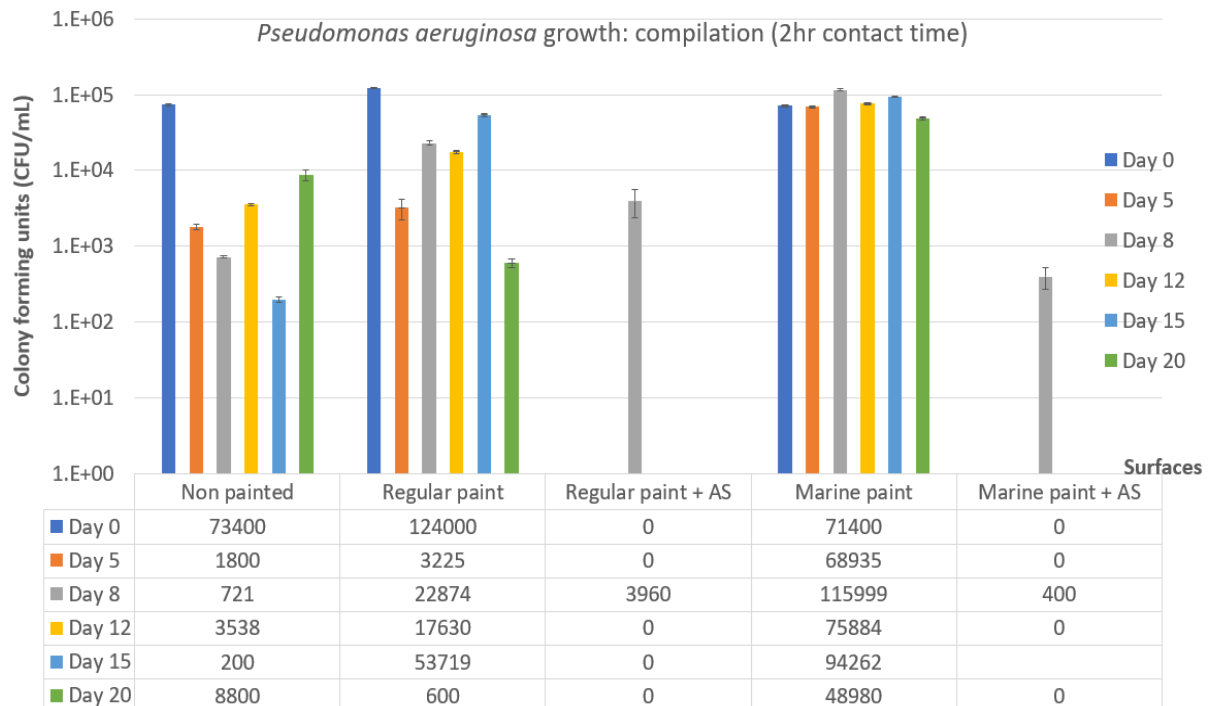


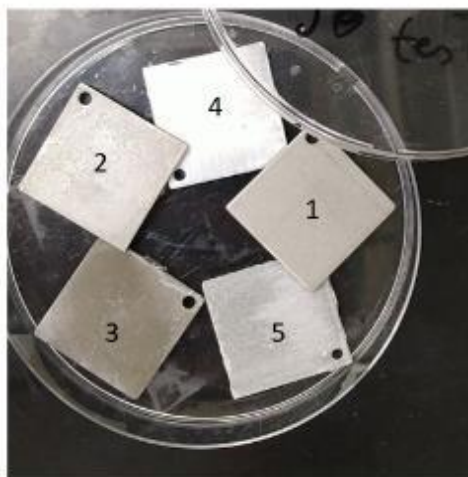
Figure 3. 3: *Pseudomonas* growth after immersing the samples in artificial ocean water

Figure 3.3 shows the bacterial colony forming units count from the agar plates for the corresponding surfaces and it was observed that the samples which has Aereus shield particles showed 100% bacterial reduction on all the days (day 8 was an outlier and this possibly could be because of cross contamination from stacked agar plates). It was also observed that the non-painted samples which were supposed to act as control showed better antimicrobial activity than the regular painted samples because of some exposed metal particles in the former.

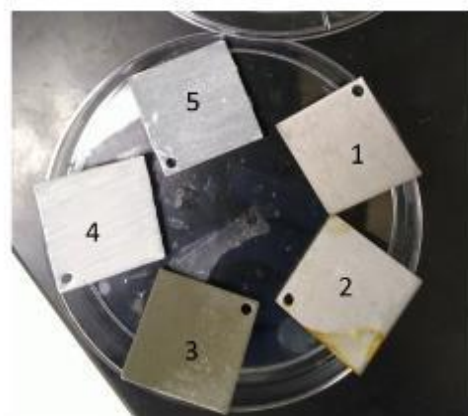
3.3.2.1 Images of the test surfaces

Figure 3.4 shows the state of the samples that were taken out periodically from the tank to observe any visible corrosion. The samples are numerically labelled and the legend is as follows (this labelling legend is followed throughout this chapter):

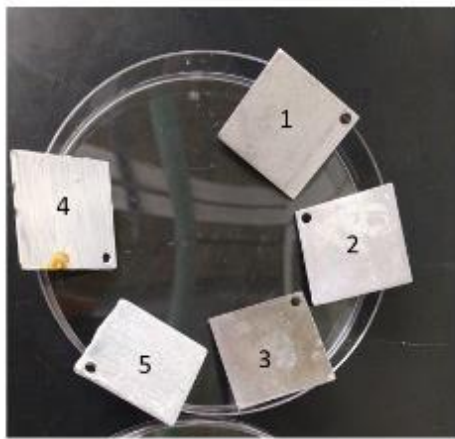
1. Non painted
2. Regular painted
3. Regular paint + Aereus shield particles
4. Marine painted
5. Marine paint + Aereus shield particles



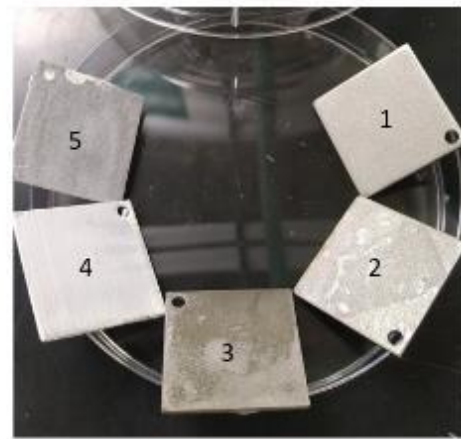
Day 0



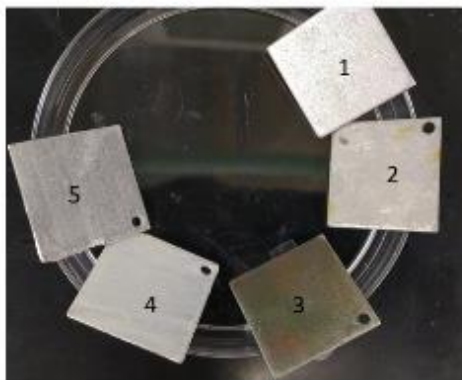
Day 5



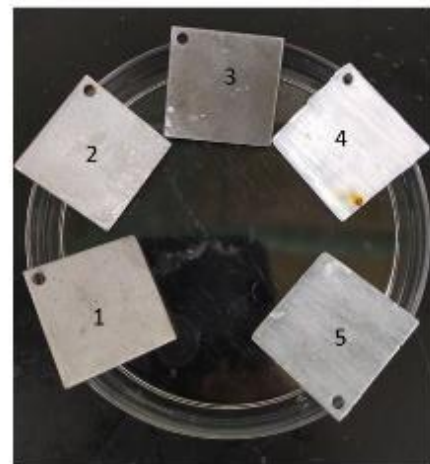
Day 8



Day 12



Day 15



Day 20

Figure 3. 4: Test surfaces post immersion in artificial ocean water

From Figure 3.4, it was observed that visible changes were observed only on samples with regular painted and regular paint + Aereus shield particles (2 and 3). Only the samples with regular paint + Aereus shield particles showed gradual worsening of physical appearance although it retained its antimicrobial efficacy over the days.

3.3.2.2 Microscope images of the test surfaces

A Dino-Lite digital microscope was used to capture the microscopic images of the test surfaces.

Day 0



Day 5



Day 8



Day 12



Day 15



Day 20



Figure 3. 5: Microscopic images (200x) of test surfaces post immersion in artificial ocean water

From Figure 3.5, it was noted that the Aereus shield particles were spread on the regular paint + Aereus shield particles and marine paint + Aereus shield particles surfaces and the gradual rust formation on regular paint + Aereus shield particles samples was observed. Fine metallic particles were observed on the non-painted samples, which may have attributed towards its better antimicrobial properties than the regular painted ones which does show any exposed metallic particles on the surface. It can also be seen that the marine painted samples have plain morphology and don't have any exposed particles which is why these samples showed poor antimicrobial properties; also, it is because of the marine paint that the samples coated with them didn't show any kind of surface corrosion.

3.3.3 Artificial sweat experiment

The same 20-day experiment was performed with the Aereus samples after changing the contents in the fish tank from artificial ocean water to artificial sweat and the antimicrobial efficacy results were noted.

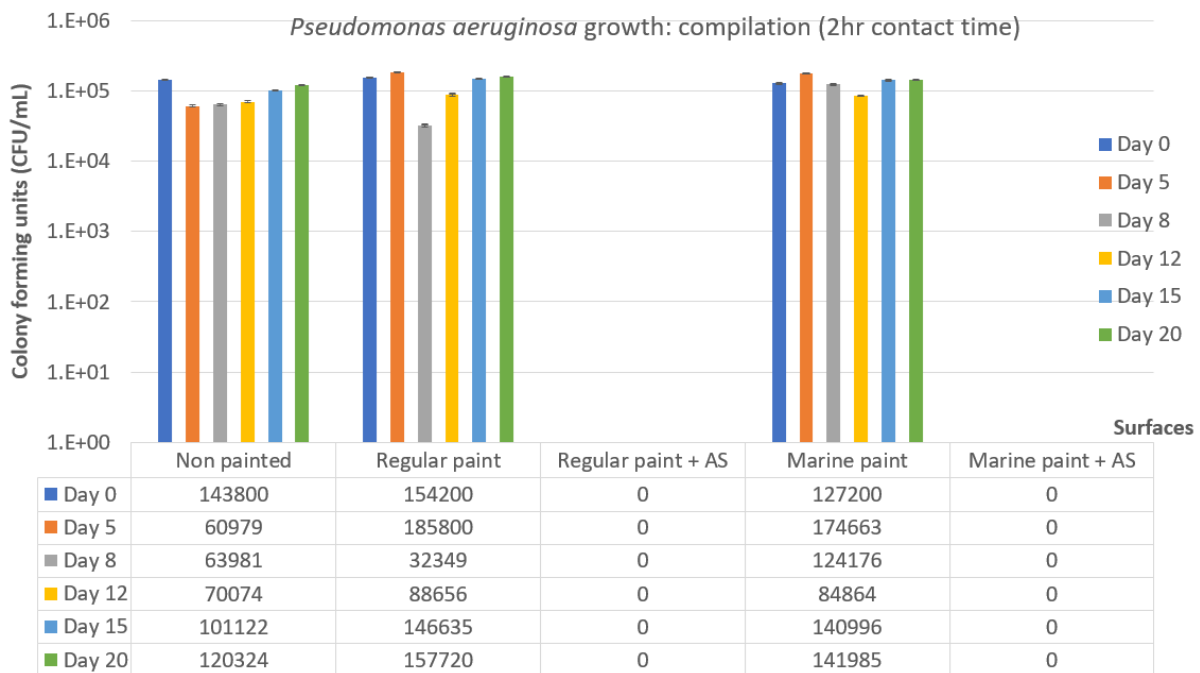
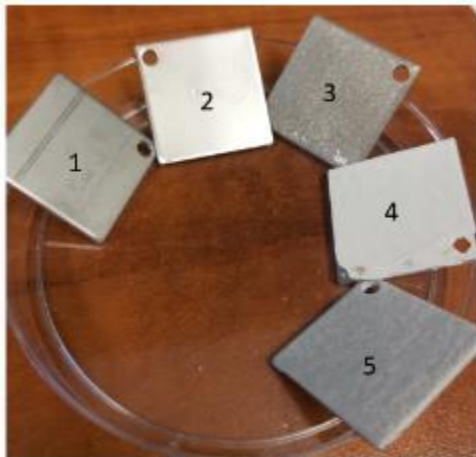


Figure 3. 6: *Pseudomonas* growth after immersing the samples in artificial sweat

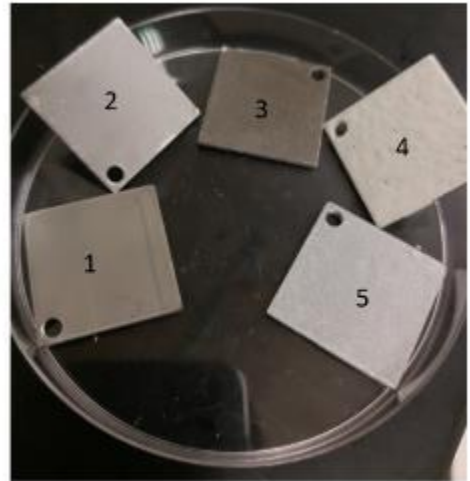
From Figure 3.6, it is observed that the trend with artificial sweat experiment was similar to the artificial ocean water experiment. The above graphs show the bacterial colony forming units count on

the surfaces and it was observed that the samples that had Aereus shield particles showed 100% bacterial reduction on all the days.

3.3.3.1 Images of the test surfaces



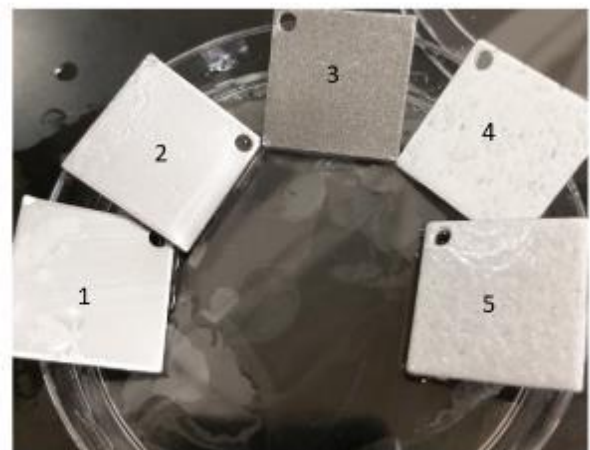
Day 0



Day 5



Day 8



Day 12

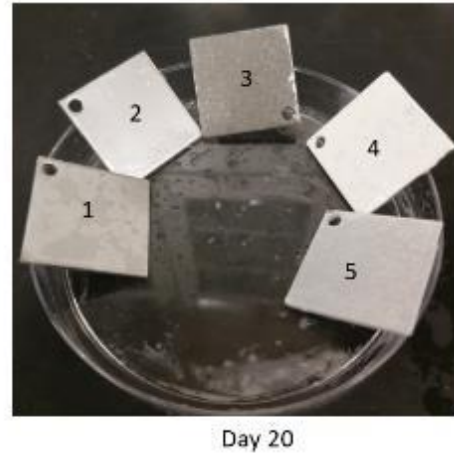
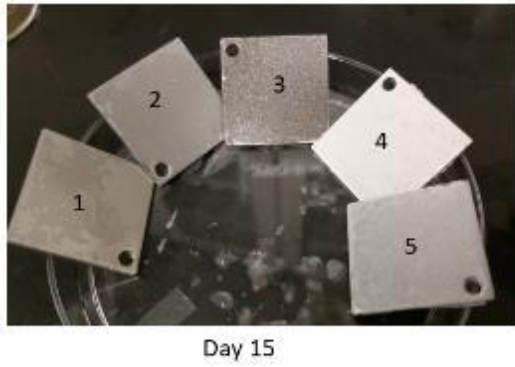


Figure 3. 7: Test surfaces post immersion in artificial ocean water

From Figure 3.7, it was observed that not much visible change was observed on most samples. Only the samples with regular paint + Aereus shield particles showed mild rusting on the surface although they retained antimicrobial efficacy over the days.

3.3.3.2 Microscope images of the test surfaces

Day 0



Non painted



Regular painted



Regular paint (with AS particles)



Marine painted



Marine paint (with AS particles)

Day 5



Day 8



Day 12



Day 15



Day 20



Figure 3. 8: *Microscopic images (200x) of test surfaces post immersion in artificial sweat*

Same trends as observed from Figure 3.5 was noted in Figure 3.8 as well, with good particle exposure only on samples containing the Aereus shield particles and the gradual formation of rust on regular paint + Aereus shield particles. But unlike the samples used in the artificial ocean water experiments, the non-painted samples did not have any exposed fine metallic particles, thus making the non-painted samples used in this experiment a better negative control. It can also be noted that the regular and marine painted samples have plain morphology and don't have any exposed particles which is why these samples showed poor antimicrobial properties, but it is only because of these paints that no visible surface corrosion was observed on the samples coated with them.

3.3.4 Hydrogen peroxide experiment

To check the antimicrobial efficacy of the samples after being in accelerated oxidizing conditions, the samples that were immersed in the artificial ocean water conditions for 20 days were then immersed in different concentrations of hydrogen peroxide (1%, 10% and 30%) for 2 hours and 24 hours after which the same EPA protocol was followed for testing the antimicrobial efficacy.

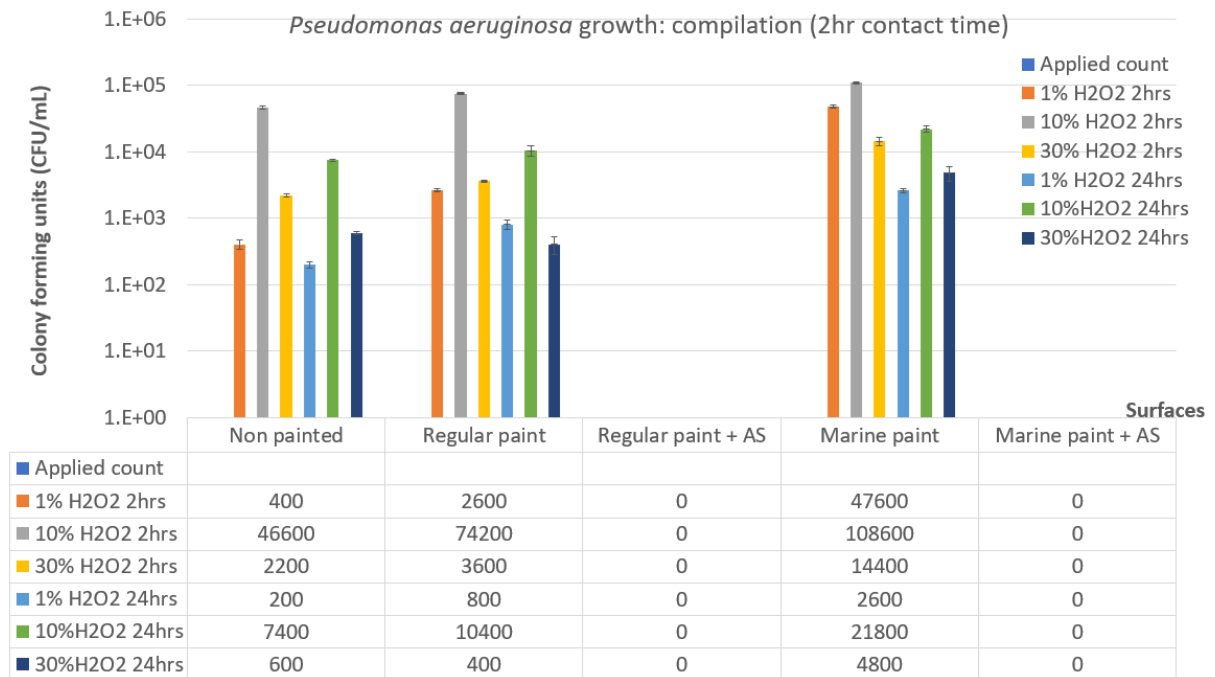


Figure 3. 9: *Pseudomonas* growth post immersion in hydrogen peroxide

From Figure 3.9, it can be concluded that even after extensive oxidizing conditions the regular painted and the marine painted samples that contained Aereus shield particles showed 100% bacterial reduction even though there was partial removal of particles from the surface by hydrogen peroxide.

3.3.4.1 Images of the test surfaces

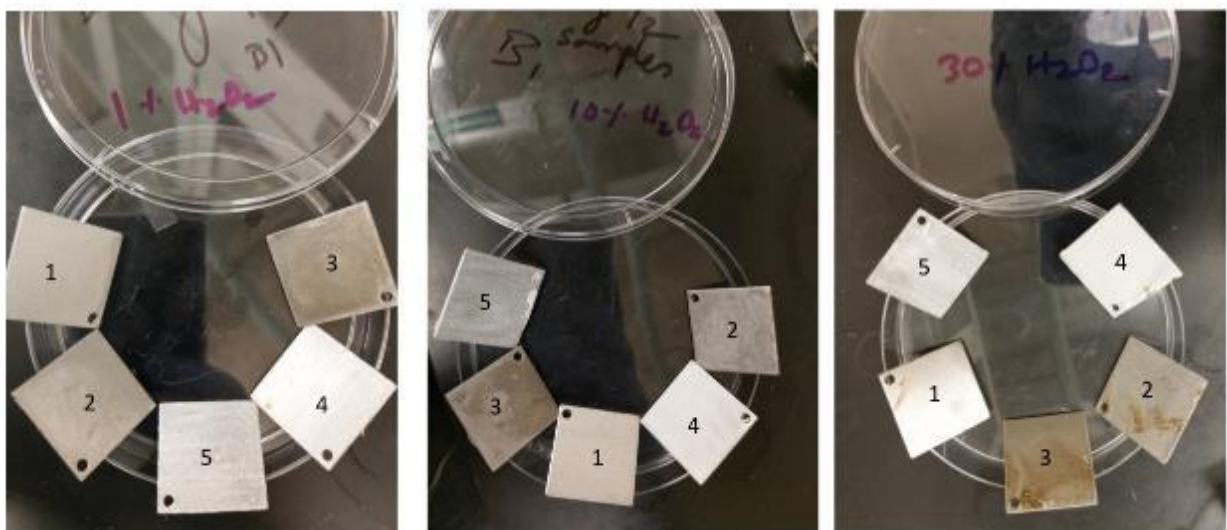


Figure 3. 10: Samples immersed in different concentrations (1%, 10% and 30%) of hydrogen peroxide for 2 hours.



Figure 3. 11: Samples immersed in different concentrations (1%, 10% and 30%) of hydrogen peroxide for 24 hours

From Figure 3.10 and 3.11, it was observed that corrosion immersion in 1% hydrogen peroxide did not induce any corrosion on the samples but when the concentration was increased to 10% and 30%, visible corrosion was noted only on regular painted samples with Aereus shield particles (sample #3).

3.3.4.2 Microscopic images of test surfaces

Day 0



1% hydrogen peroxide for 24 hrs



10% hydrogen peroxide for 24 hrs



30% hydrogen peroxide for 24 hrs



Figure 3. 12: *Microscope images (200x) of the test surfaces after being immersed in different concentrations of hydrogen peroxide for 24 hrs*

From Figure 3.12, it was observed that hydrogen peroxide apparently dissolved some of the Aereus shield particles from the regular painted and marine painted samples that were coated with Aereus particles rendering few spots on the surface without any exposed particles. However, most of the particles remained intact even after being immersed in higher concentrations of hydrogen peroxide for 24 hrs which accounted for the retention of the antimicrobial efficacy even after going through accelerated oxidizing conditions.

3.4 Conclusion

Experiments were conducted on the marine painted samples provided by Aereus Technologies Inc. in both gradual oxidizing conditions (artificial ocean water and artificial sweat in fish tank) and rapid oxidizing conditions (different concentrations of hydrogen peroxide for 2 hrs and 24 hrs respectively) and it was observed that in either case the regular painted and the marine painted samples containing Aereus shield particles did not show any kind of bacterial growth even after performing experiments in extensive test conditions. The samples with regular paint and Aereus shield particles did show some corrosion whereas the samples with marine paint and Aereus shield particles did not corrode. Although, when immersed in higher concentrations of hydrogen peroxide, the Aereus shield particles attached to the surface started to detach, but since it was just a small amount and most of the particles

remained intact, the antimicrobial efficacy of the regular painted and marine painted samples with Aereus shield particles were retained and 100% bacterial reduction was observed. Thus, we can conclude that regular painted and marine painted samples with Aereus shield particles have the ability to survive harsh oxidizing conditions without losing their antimicrobial properties.

Chapter 4

Recyclable antimicrobial sulphonated poly (ether ether ketone) - copper films: flat vs micro-pillared surfaces

4.1 Introduction

With the characterization skills and work experience gained from working on the industrial project, the synthesis and investigation of using sulphonated poly (ether ether ketone) (SPEEK) – copper film as an effective alternative antimicrobial material is investigated and reported in this chapter. A novel method was applied in this work which involves solution casting SPEEK films in polydimethylsiloxane (PDMS) negative micro-pillar mold. This method produces films with micro-pillars on the surface which exposes the copper micro-particles to a greater extent on the surface. The differences in the level of copper micro-particle exposure, other physical and chemical properties and antimicrobial properties were systematically studied by making a direct comparison to the flat films.

4.1.1 Solution casting

Polymer solution casting is a technique that can replace film extrusion to deliver high-quality films with superior optical, mechanical and physical film properties.

In polymer solution casting, polymer is dissolved or dispersed in solution, coated onto a carrier substrate, and then the water or solvent is removed by drying to create a solid layer on the carrier. The resulting cast layer can be stripped from the carrier substrate to produce a standalone film. Before or after stripping, the cast film can be laminated with other webs or coated with other materials to create multi-layer products.

Manufacturing process advantages of polymer solution casting over traditional film extrusion methods include:

- Processing at low temperatures, which is valuable for thermally activated films or applications incorporating temperature-sensitive active ingredients
- Ability to produce high-temperature resistant films from non-thermoplastic but soluble raw materials

- Simplified incorporation of additives and fillers
- Single pass manufacturing of multi-layer films (*i.e.* the ability to cast a free film, then coat an adhesive and laminate release liner on one side and coat a top coat on the other side)
- Wider range of material choices with casting from either aqueous or solvent-based solutions

Advantages of the resulting film include:

- Greater film thickness uniformity, as tight as +/-2%
- Wider range of film thickness, from 150 microns down to less than 12 microns
- Films that are gel and pinhole free
- Excellent flatness and dimensional stability
- Isotropic orientation (mechanical and optical) as film is not stretched during manufacture
- Absence of typical extrusion process lubricants

The suitability of polymer solution casting is evaluated on a case-by-case basis according to the product application, base material, intended use and numerous other considerations. The primary materials employed in preparation of the film by solution casting were SPEEK, copper and NMP. These were the essential materials required in solution casting of the films which is the method we followed to obtain the films. NMP was used as the solvent in the solution casting process for all the films prepared.

4.2 Materials and methods

4.2.1 Materials used

Poly(ether-ether-ketone) powder (PEEK) (mean particle size 80 micron, Goodfellow), sulphuric acid (ACS Reagent 95-98%, Sigma-Aldrich), N-Methyl-2-pyrrolidone (NMP) (ACS Reagent, ≥99%, Sigma-Aldrich), Copper microparticles (spheroidal, 10-25 µm, 98%, Sigma-Aldrich), Sylgard™ 184 silicone elastomer kit (Dow Corning), Gecko tape (Setex Dry Adhesive® 912C, nanoGriptech®), Plate Count Agar (PCA) (Standard methods agar, Difco™) and Potato Dextrose Agar (Difco™).

4.2.2 Fabrication of SPEEK

PEEK (3 g) was added in a round bottom flask and 35 mL of sulphuric acid was added to it. The solution was maintained at 45°C for 24 hours under gentle stirring through the use of a silicone oil bath. The solution was then slowly added to an excess of chilled de-ionized water (0°C), quenching the reaction. The SPEEK was then continuously washed with chilled de-ionized water till it was pH neutral. The final product was stored in a petri-dish after drying in room temperature [140].

4.2.2.1 Degree of Sulphonation

SPEEK (0.1 g) was soaked in 4 mL of 0.1M NaCl solution for 24 hours and then back-titrated with 0.1 M NaOH solution using phenolphthalein as indicator until the turned from colourless to pink.

Ion Exchange Capacity (IEC),

$$\text{IEC} = \frac{\text{Volume of NaOH} \times \text{molar concentration of NaOH}}{\text{weight of SPEEK}}$$

Degree of Sulphonation (DS),

$$(\text{DS}) = \frac{M_p \times \text{IEC}}{(1000 - (M_{\text{SO}_3\text{H}} \times \text{IEC}))} \times 100$$

where, $M_p = 288$ g/mol and $M_{\text{SO}_3\text{H}} = 81$ g/mol are molecular weights of PEEK monomer unit and sulphonic acid group respectively [141].

$$\text{IEC} = \frac{1.2 \text{ mL} \times 0.1}{0.1 \text{ g}} = 1.2 \text{ meq g}^{-1}$$

$$\text{DS} = \frac{288 \times 1.2}{1000 - (81 \times 1.2)} \times 100 = 38.28\%$$

We intended to make an antimicrobial material which swells less in water and it was suggested from literature that lower DS SPEEK swells less than higher DS SPEEK [142]. As it was difficult to

dissolve SPEEK with very low DS in the solvent, a range between 35-40% was preferred and as a result of which SPEEK with DS 38.28% was used for the entirety of this research.

4.2.3 Synthesis of PDMS negative micro-pillar mold

Gecko tape was cut into 2.5 cm x 2.5 cm square and adhered to a metal mold. Sylgard™ 184 silicone elastomer kit has DOWSIL™ 184 silicone elastomer base and curing agent. Polydimethylsiloxane (PDMS) 10:1 was formed by mixing 20 g of part A (elastomer base) with 2 g of part B (curing agent). The PDMS prepolymer was then poured into the mold and held under vacuum until there were no visible bubbles. It was then cured at 80°C for 1 hour. The resulting PDMS negative micro-pillar mold was then carefully demolded from the metal mold.

4.2.4 Solution casting of SPEEK and SPEEK-Cu flat films

SPEEK (1 g) was added to a glass vial and dissolved by ultrasonication with 3 mL of the solvent NMP. The dissolved contents were then added dropwise homogeneously on a levelled glass petri-dish, vacuumed and left in an oven overnight at 120°C for the solvent to evaporate. The resulting film was then carefully peeled off from the petri-dish. The same procedure was applied for synthesizing SPEEK – Cu films with the only difference being the addition of copper microparticles (20 mg, 2% by weight), into the SPEEK dissolved in NMP (1 g in 3 mL).

4.2.5 Solution casting of SPEEK and SPEEK-Cu micro-pillar films

SPEEK (1 g) was added to a glass vial and dissolved by ultrasonication with 3 mL of the solvent NMP. 1.5 mL of the solution was poured into the PDMS negative micro-pillar mold. The mold was then held under vacuum till no bubbles were visible. The NMP was then evaporated in an oven overnight at 120°C. The resulting film was then carefully peeled off from the mold. The same procedure was applied for synthesizing SPEEK-Cu films but with the addition of copper microparticles (2 mg, 2% by weight), into the SPEEK dissolved in NMP (1 g in 3 mL). Figure 4.1 illustrates some of the sample images.

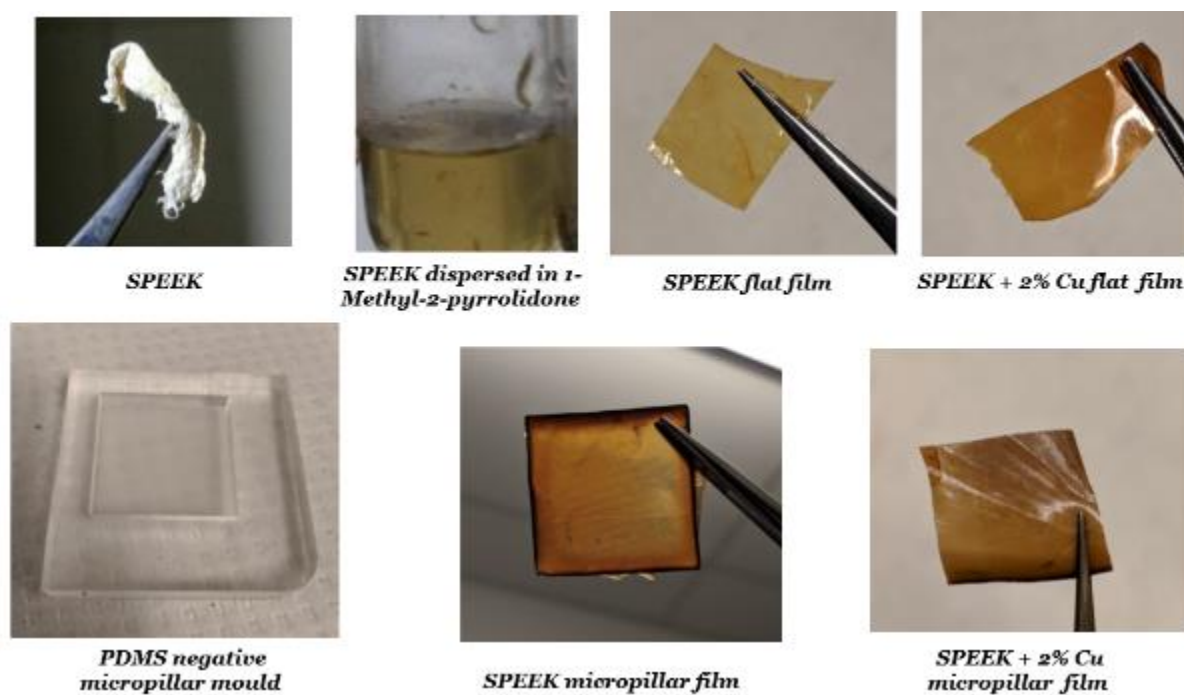


Figure 4. 1: Sample images

4.2.6 Preparation of Plate count Agar (PCA) and Potato Dextrose Agar (PDA) plates

For *E. coli* assays, purchased PCA powder (23.5 g) was added to a 2 litres conical flask and one litre of de-ionized water was added. The contents were mixed, autoclaved at 121°C for an hour after which they were transferred into petri dishes and cooled to room temperature. The same procedure was followed to prepare PDA plates for *Aspergillus niger* and *Saccharomyces cerevisiae* (yeast) assays, but with 39 g of PDA in one litre of deionized water.

4.2.7 Environmental Scanning Electron Microscope (ESEM)/Energy Dispersive X-ray Spectroscopy (EDS) and water contact angle analysis

ESEM/EDS and water contact angle analysis were performed for both the flat and micro-pillared films. The test samples were cut into small pieces and pasted on a carbon tape which was then pasted on an aluminium stub. The test was then performed using FEI Quanta Feg 250 ESEM (with EDX) at low pressure (~0 bar) and the results were recorded. Water contact angles were measured using a custom-built goniometer. Water (3 μL) was placed on sample using a syringe pump after which an image was captured using a fixed focal length high magnification camera with a backlight. The captured image was then analyzed using a MATLAB script to determine the contact angle.

4.2.8 Tensile strength measurements

Tensile strength tests are predominantly performed on the materials to determine how they will behave under tension load. In this test, the material sample is subjected to constant and controlled tension/stress until failure. The data obtained from the test is then analysed to determine the ultimate tensile strength.

The samples of dimensions 2.5 cm x 2.5 cm were held in place by tight clamps in the universal macro-tribometer (UNMT-2MT, T1377 by Centre for Tribology, Inc.) after which stretch load was applied until fracture point (permanent deformation) using 100 kg load cell with no heat at room temperature. The stress and strain data were collected using the universal macro tribometer's system software, with which the tensile strength of the material was analysed and stress vs strain curves were plotted.

4.2.9 Thermogravimetric analysis (TGA) and Differential Scanning Calorimetry (DSC)

TGA is a destructive characterization technique (because often it requires the sample to be heated until it decomposes into its constituents) in which the mass of the sample is measured over time as the temperature changes whereas DSC can either be a destructive or non-destructive method of characterization depending on the range of operation and it is a technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. DSC is characterization technique that is predominantly used to determine the glass transition, crystallization temperature and melting point of the material.

A typical TGA and DSC setup consists of a precision balance, sample pan and a programmable furnace with a high precision thermocouple. In both the techniques, a sample pan is weighed using the precision balance whose mass is then input in the computer program to record. Then the sample is placed on the pan and loaded into the equipment for the characterization to begin. Minimal sample masses (> 1 mg) are needed for processes. So, the film samples that were to be tested were cut into small pieces (whose mass were ~ 3-5 mg for TGA and ~ 1-2 mg for DSC) before loading them for characterization. TGA was carried out (using TA instruments – TGA Q500 V20.13 Build 39) until 800°C with a ramp of 10°C/min and DSC was performed on the samples (using TA instruments – DSC

Q2000 V24.11 Build 123) with a heat flow ramp of 10°C/min from 30°C to 200°C to determine their glass transition temperatures (T_g). Both the characterization techniques require little to no sample preparation with the only important step being the cleaning of the sample pan to remove any foreign substances that can interfere.

4.2.10 Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) analysis

For examining the copper ion release, SPEEK + 2% Cu (flat and micro-pillared) films 2.5 cm x 2.5 cm were immersed in 100 mL de-ionized water separately for 10 days. The water samples were periodically collected and slightly acidified using concentrated nitric acid (~ pH of 2.3) after which they were transferred into ICP tubes and tested for copper content using ICP-OES equipment (Prodigy ICP, Teledyne).

4.2.11 Protocol for antimicrobial efficacy tests [139]

The SPEEK and SPEEK - Cu films were washed using de-ionized water and let dry in room temperature prior to performing the antimicrobial efficacy tests. A test culture of *E. coli*, *Saccharomyces cerevisiae* (yeast) and *Aspergillus niger* in deionized water was prepared separately. The target level on the stainless-steel control after 60 minutes was 5×10^5 to 5×10^6 CFU/mL, so an applied load of approximately 10^8 CFU/mL was found to be satisfactory in preliminary testing. For each sample, 20 μ L of culture was applied and spread with the sterile pipette tip or sterile loop/spreader over an area of approximately 2.5 cm x 2.5 cm. The samples were then kept in a humid environment to minimize the evaporation of the applied culture. At the end of a stipulated time (15, 60 minutes for bacteria and 120 minutes for fungi in this experiment accordingly), the test surfaces were rinsed with 20 mL of sterile de-ionized water captured in a beaker. The contents in the beaker along with the samples were sonicated at a frequency of 60Hz for 5 minutes assist in recovery of the bacteria from the test surfaces to the deionized water. After sonication, 100 μ L and 10 μ L of the contents from the beaker were plated in separate PCA or PDA plates for bacteria or fungi, respectively. These plates were then incubated at 36°C for 24 or 48 hours for bacteria and fungi, respectively. The visible microbial colonies were then counted and recorded.

4.3 Results and discussion

Since the approach for film synthesis involves dissolving SPEEK in an organic solvent and then dispersing copper microparticles by ultrasonication in the contents before transferring it into different molds (PDMS negative micro-pillar and glass petri-dish), variations in surface morphology are observed. This variation in the surface texture of flat and micro-pillared films observed as a result of a different fabrication process is illustrated as in Figure 4.2. It was observed that localized aggregation of copper micro-particles in a specific region occurs in flat films while they move together during the process of solvent evaporation. Other than this, the flat films had no distinguishable surface feature as compared to the micro-pillared films. The PDMS negative micro-pillar mold forms mushroom micro-pillars on the surface of the film which increases the water contact angle thus rendering the film hydrophobic through the formation of a Cassie-Baxter state (with increasing surface roughness, a composite interface is formed as a result of trapped vapour pockets underneath the liquid; this heterogenous wetting is usually described by the Cassie-Baxter model). [143], [144]. Vacuum casting the solution allows for accurate positive structures as well as exposing copper particles due to the pressure gradient existing between the surface of the mold and the bottom of the negative micro-pillar cavity. This exposes copper particles on the surface via the micro-pillars after solvent evaporation.

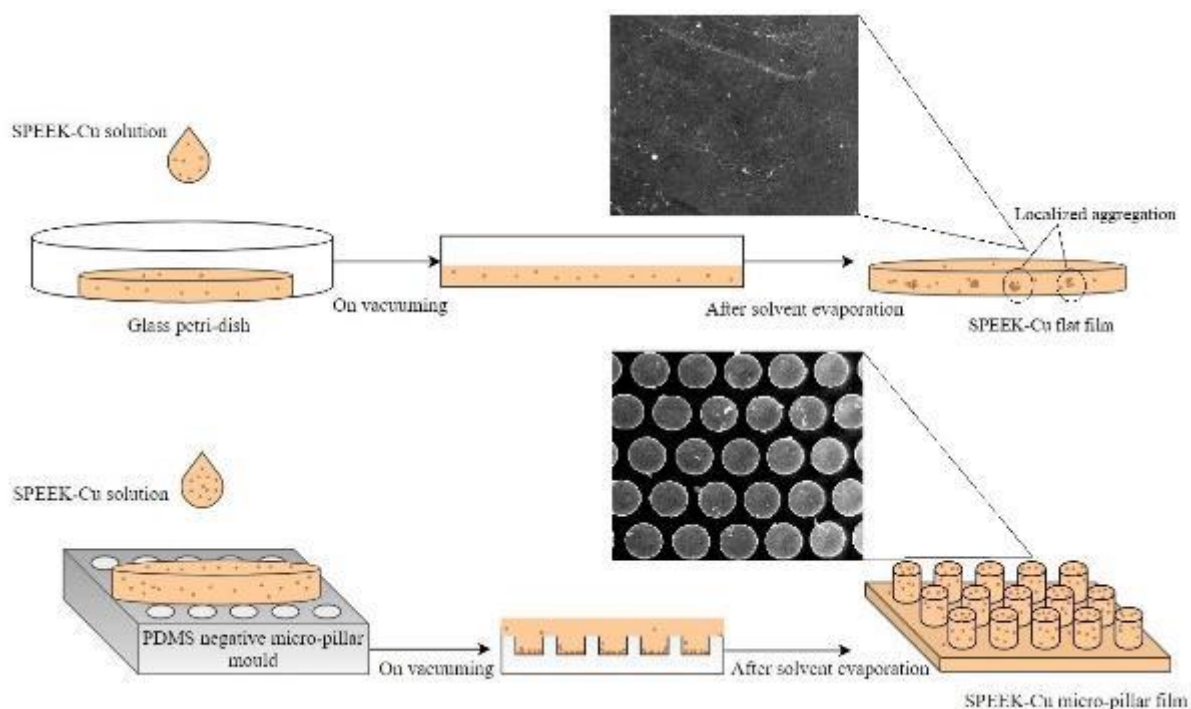


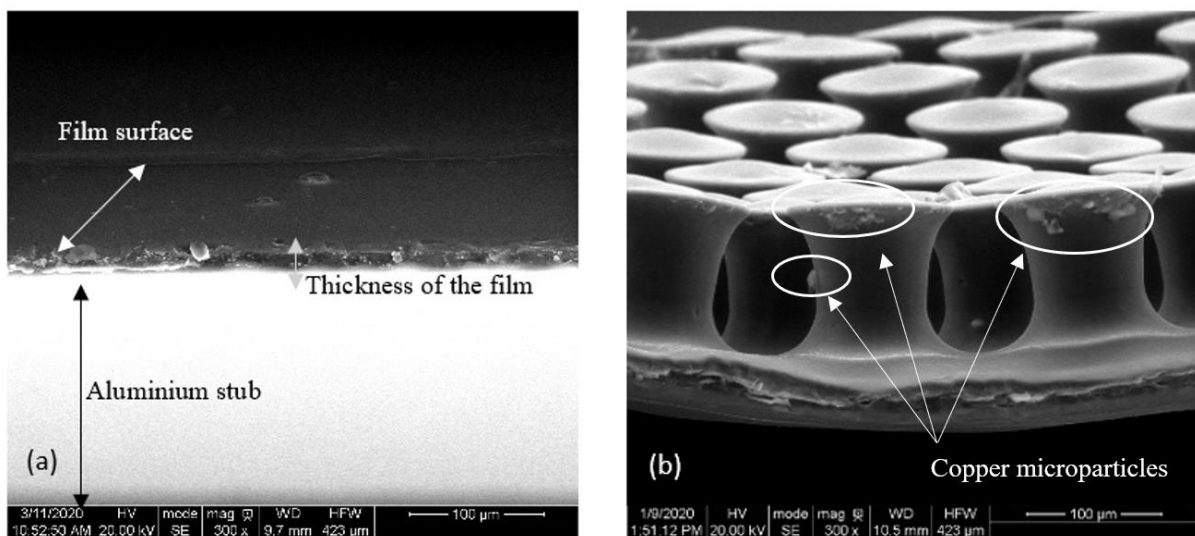
Figure 4. 2: Schematic representation of the fabrication process of SPEEK-Cu films and microscopic images (150x) of the surface (top view)

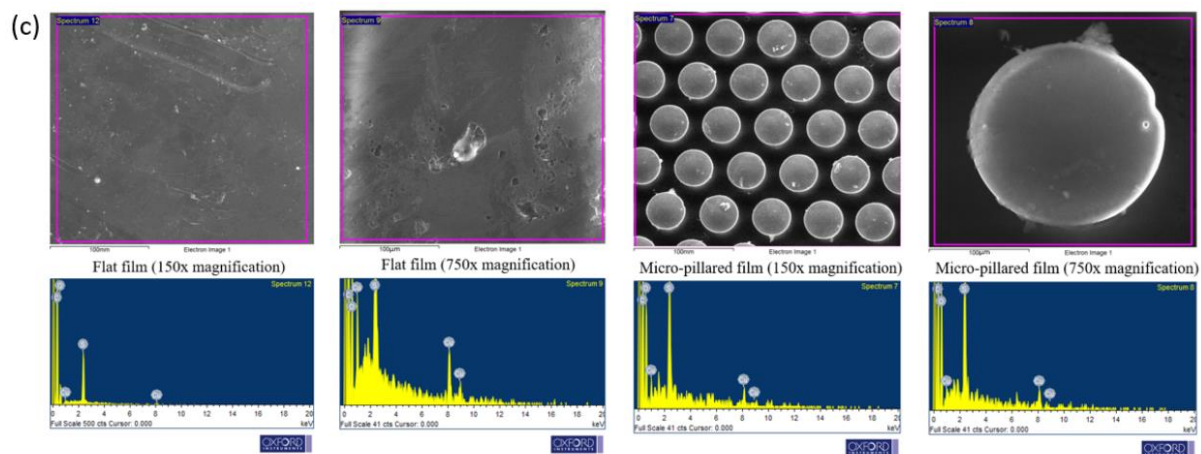
4.3.1 ESEM/EDS and water contact angle analysis

ESEM/EDS and water contact angle analysis were performed for both the flat and micro-pillared films. The prepared SPEEK and SPEEK – Cu micro-pillars were 117 μm in height, 125 μm in top diameter with 155 μm centre to centre spacing. From Figure 4.3 (a) and 4.3 (b) it can be observed that the SPEEK + 2% Cu flat film did not have any exposed copper micro-particles on the surface whereas the micro-pillared film had copper micro-particles attached to the micro-pillars leaving them exposed. The better copper micro-particle exposure in the SPEEK micro-pillars happened as a result of the denser copper particles sinking into the mold cavity under vacuum, which leads to their exposure during the subsequent evaporation of the solvent. From the EDS analysis in Figure 4.3 (c) it can be observed that localized aggregation of copper micro-particles occurs in SPEEK + 2% Cu flat film. It is evident by the presence of 4.10 wt. % copper in the 750x-magnified flat film as compared to 0.86 wt. % copper in the broader image i.e. 150x-magnified flat film. Whereas, an almost uniform dispersion of copper micro-particles and better exposure in the larger picture is observed in the micro-pillared films. It is corroborated by the presence of 2.11 wt. % copper in 750x-magnified micro-pillared film which is

close to the observed 2.50 wt. % copper in the broader image 150x-magnified micro-pillared film. From these results it was concluded that micro-pillared films exposed more copper micro-particles on the surface whose distribution was also more uniform than flat films and also prevented localized aggregation due to geometrical constraints.

From Figure 4.4 (a) and 4.4 (b) it can be observed that the flat film and micro-pillared film have a water contact angle of 64° and 131° respectively. The surface structure modification led to the formation of a Cassie-Baxter interface making micro-pillars significantly more hydrophobic than the flat surface [144] [143]. As discussed briefly in Chapter 2 under section 2.1, this is a favorable property for an antimicrobial surface as it would potentially prevent bacterial adhesion and biofilm formation and also possible enable efficient surface cleaning. Further testing should be done to quantify bacterial adhesion to the micro-pillar tops as the diameter of the top is around $125\ \mu\text{m}$, and bacteria are typically around $1\ \mu\text{m}$, so it is possible to have dozens or hundreds of bacteria on such a surface.





Element	Flat film (150x magnification)		Flat film (750x magnification)		Micro-pillared film (150x magnification)		Micro-pillared film (750x magnification)	
	Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %
Carbon	72.34	80.02	68.84	78.30	72.25	80.27	66.56	75.16
Oxygen	20.91	17.36	21.71	18.54	20.61	17.19	26.40	22.38
Sulphur	5.90	2.44	5.35	2.28	5.04	2.1	4.54	1.92
Copper	0.86	0.18	4.10	0.88	2.11	0.44	2.50	0.53

Figure 4. 3: ESEM images of (a) sideview of SPEEK + 2% Cu flat film (b) sideview of SPEEK + 2% Cu micro-pillared film (c) EDS analysis for SPEEK + 2% Cu films

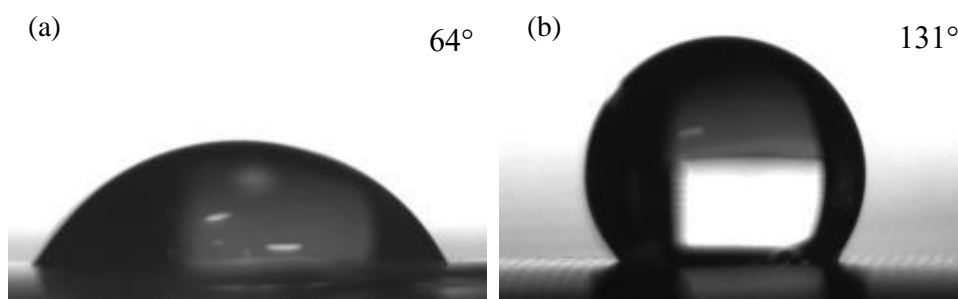


Figure 4. 4: Water contact angle (150x magnified) of (a) flat film (b) micro-pillared film

4.3.2 Tensile strength measurements

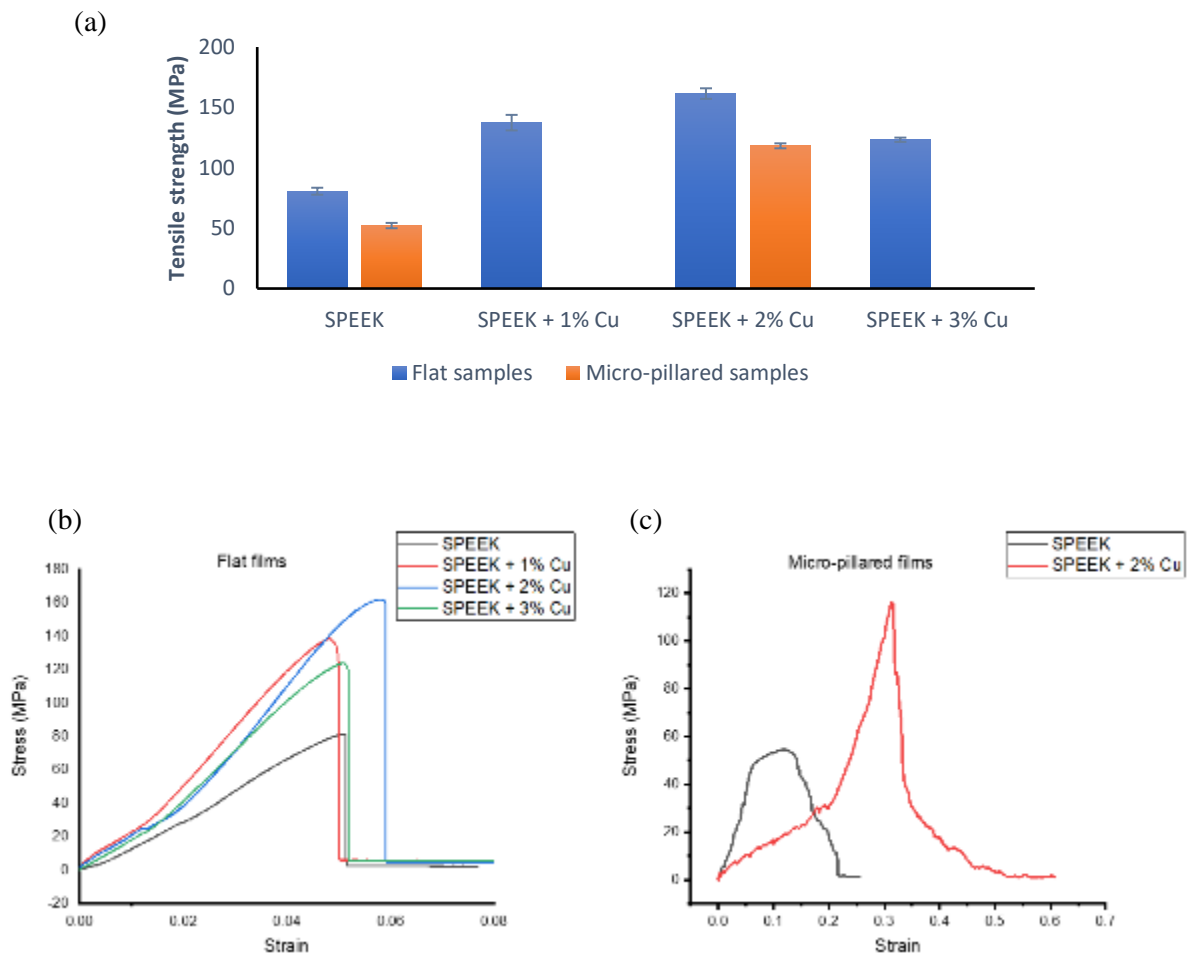


Figure 4. 5: (a) Tensile strength measurements (b) Stress-strain curves for flat films (c) Stress-strain curves for micro-pillared films

Initial tests were conducted with flat films to find out the suitable weight percent of copper micro-particles that can be added to the polymer without compromising its tensile strength. The tensile strength tests were performed on three different samples of the same kind, the standard deviation of which are plotted as error bars in Figure 4.5 (a). As illustrated in Figure 4.5 (a) it was found that the tensile strength of the SPEEK flat film increased from 80 MPa to 160 MPa with addition of copper micro-particles up to 2% by weight, after which the tensile strength began to drop. This is in agreement with the literature which suggests that low percentage of fillers (up to 3 weight %) act as reinforcing agents to improve the strength of SPEEK composite films and addition of high filler content (>3 weight %) would result in aggregation within the polymer matrix thus leading to reduced tensile strength [145]. A similar trend was observed with stress-strain curves for flat films as illustrated in Figure 4.5 (b) with the strain at

break increasing with addition of copper micro-particles up to 2% by weight, after which it dropped. Based on the results, 2% Cu was determined to be used in further studies providing both antimicrobial activity and strength. As a result of which only SPEEK and SPEEK + 2% Cu micro-pillared films were synthesized.

Measurements were further conducted on SPEEK and SPEEK + 2% Cu micro-pillared samples; it was observed from Figure 4.5 (c) that the micro-pillared films had slightly lower tensile strengths than the flat films with 52 MPa and 118 MPa respectively. The reduction of tensile strength and increase in strain values in the micro-pillared samples can be attributed to the change in surface structure, that is, the creation of inter-pillar spaces, which as observed live from tests imparts a good amount of stretch ability.

4.3.3 TGA and DSC analysis

TGA and DSC were performed for each of the four samples: SPEEK flat film, SPEEK + 2% Cu flat film, SPEEK micro-pillared film and SPEEK + 2% Cu micro-pillared film and the results recorded are illustrated as thermographs in Figure 4.6 and 4.7. It was observed that the trends were almost the same for all the tested samples indicating that the addition of copper micro-particles had little impact on the thermal properties. In Figure 4.6, it can be observed that all the samples exhibited a two-step thermal degradation process which was in accordance with literature. The minor weight loss below 200°C can be attributed to the removal of residual water due to the hygroscopic nature of SPEEK. There was a 20-25% weight loss at a temperature range of 200-300°C which was due to the degradation of the sulphonic acid groups in the polymer matrix. The next major weight loss started at around 500°C which was higher than the melting point of the base polymer poly (ether ether ketone) (PEEK) ($T_m = 343^\circ\text{C}$). This peak indicated the onset of polymer decomposition. These results corroborate the thermal stability of the films.

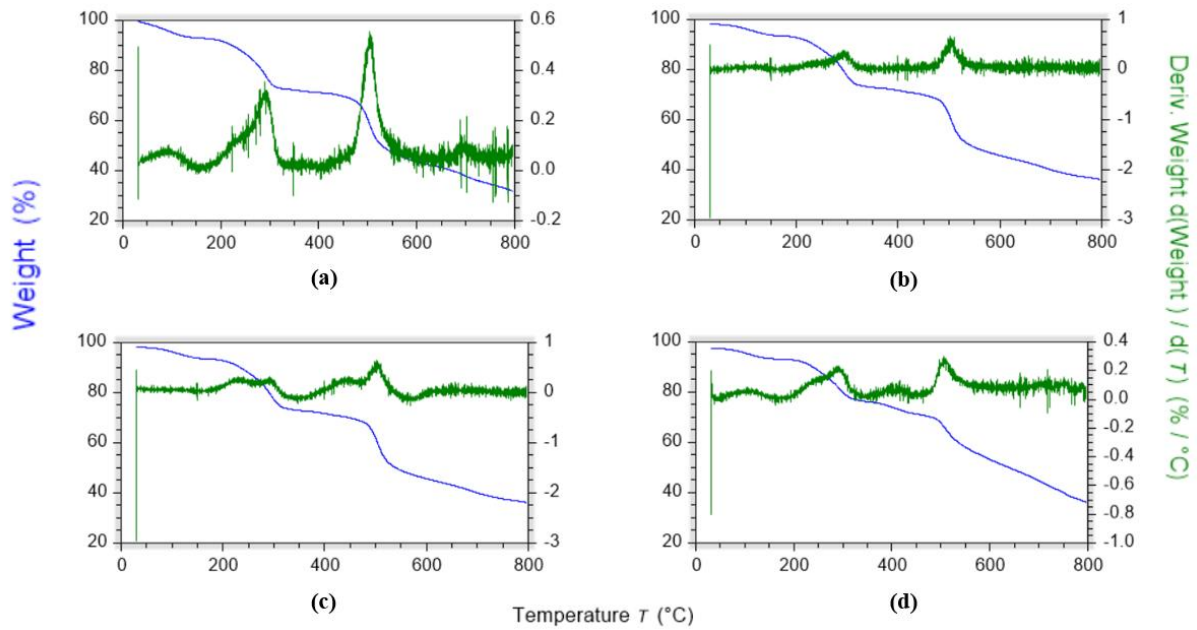


Figure 4. 6: TGA curves for (a) SPEEK flat film (b) SPEEK + 2% Cu flat film (c) SPEEK micro-pillared film (d) SPEEK + 2% Cu micro-pillared film

DSC was performed on the samples to determine the glass transition temperature (T_g) of the films. It was observed from Figure 4.7 that T_g of all the tested samples was almost the same with the onset starting at $\sim 160^\circ\text{C}$ and it was noted that this is slightly over the T_g of PEEK ($T_g = 143^\circ\text{C}$). The reason for slightly elevated T_g was the sulphonation which causes stiffness in PEEK [146] and thus an increase in the glass transition temperature compared to PEEK [147]. The increase could also be attributed to the restrained chain mobility caused by the addition of sulphonic acid group to the PEEK main chain.

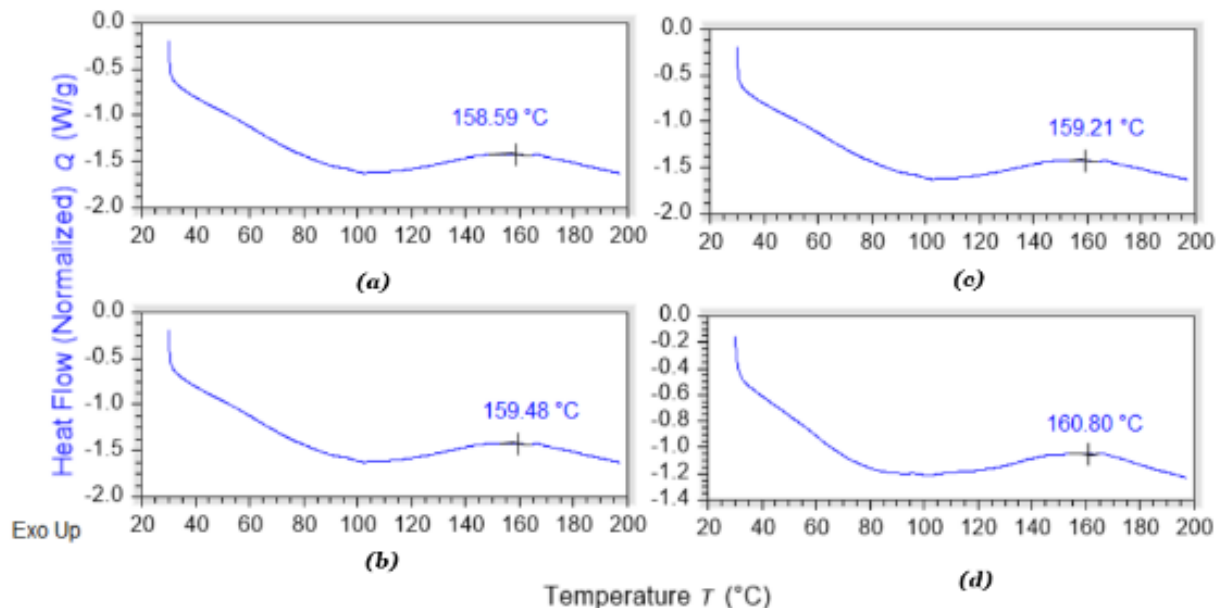


Figure 4. 7: DSC curves for (a) SPEEK flat film (b) SPEEK + 2% Cu flat film (c) SPEEK micro-pillared film (d) SPEEK + 2% Cu micro-pillared film

4.3.4 ICP-OES analysis for copper ion release

For examining the copper ion release, SPEEK + 2% Cu (flat and micro-pillared) films 2.5 cm x 2.5 cm were immersed in 100 mL de-ionized water separately for 10 days and the water samples were periodically collected and tested using ICP-OES. The recorded results were tabulated as in Table 4.1.

Table 4. 1: Copper ion release

	Copper in solution (mg/L)	
	<i>Flat sample</i>	<i>Micro-pillared sample</i>
Day 0 (DI water blank reference)	<0.005	<0.005
Day 3	<0.005	0.106
Day 5	<0.005	0.194
Day 7	<0.005	0.305
Day 10	<0.005	0.443

From Table 4.1 it was clearly observed that there was negligible copper ion release from the SPEEK-Cu flat films into the de-ionized water whereas the micro-pillared film showed progressive release. This was because of the lack of exposed copper micro-particles on the flat film as studied from the ESEM analysis in section 4.3.1 and these results confirmed the fact that micro-pillared films have exposed copper micro-particles that could contribute towards enhanced antimicrobial properties

through copper ion release. This is important as the antimicrobial activity requires the release of copper at some minimal rate [120].

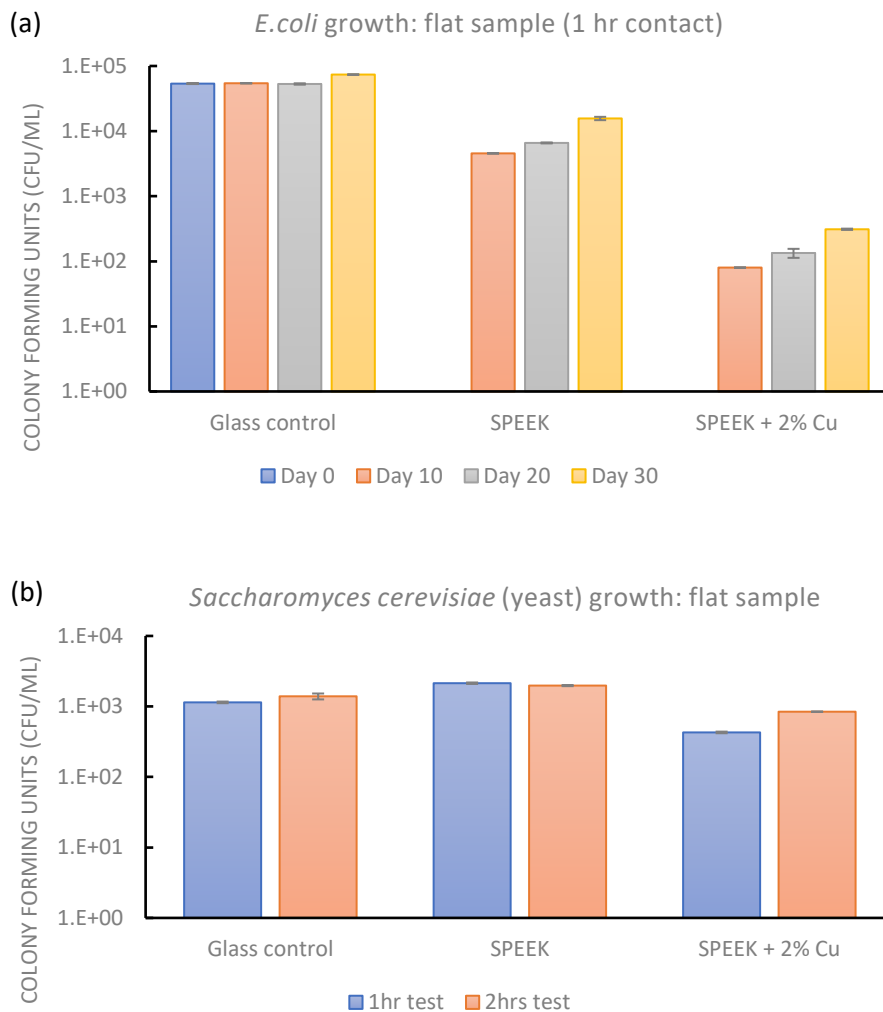
4.3.5 Antimicrobial efficacy tests

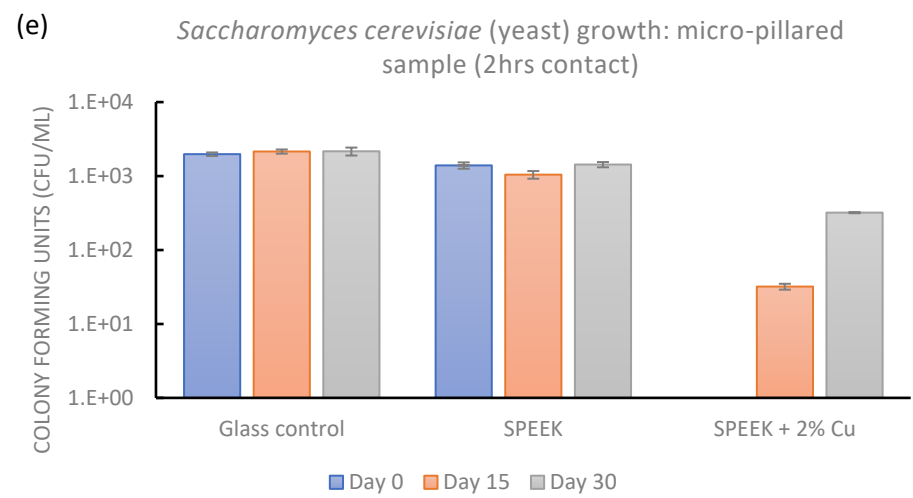
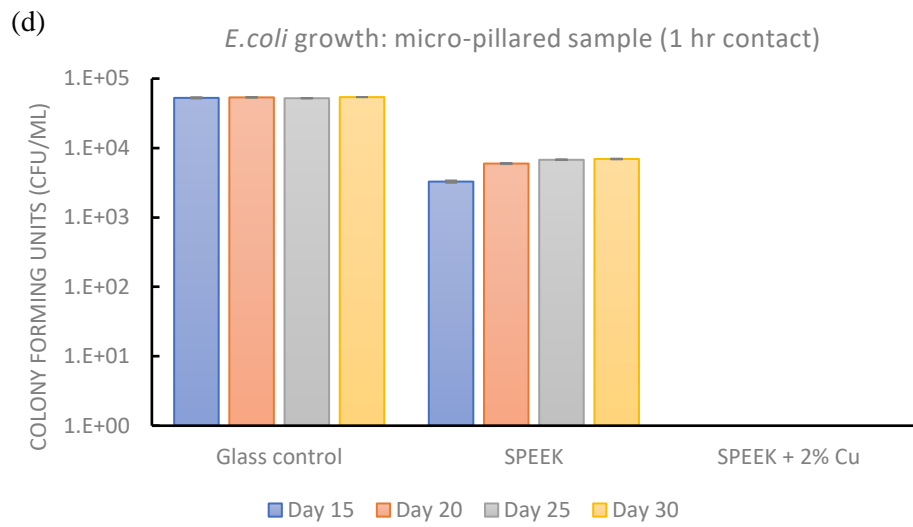
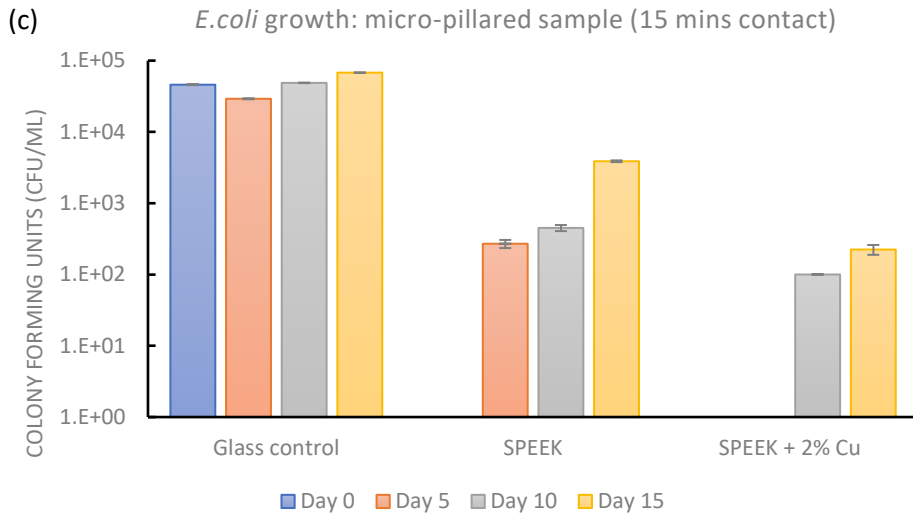
E. coli, *Saccharomyces cerevisiae* (yeast) and *Aspergillus niger* were used to carry out the antimicrobial efficacy test on both flat and micro-pillared films and the recorded results are as illustrated in Figure 4.8. Throughout the testing, a 2.5 cm x 2.5 cm cut glass microscope slide was used as a control surface with which the SPEEK and SPEEK – Cu samples were compared. The same set of samples was used for the month-long testing with a particular microorganism. Preliminary tests were performed with flat samples to set a benchmark with which the micro-pillared samples were compared. Between the tests, the samples were washed, dried and stored in a closed petri-dish which was safely placed inside a biosafety cabinet.

As shown in Figure 4.8 (a) it was observed that both SPEEK and SPEEK + 2% Cu flat films showed over 4 log reduction of *E. coli* (>99.99% killing) on day 0 (day of film synthesis) but progressively started showing reduced antimicrobial activity as the films aged. In the case of yeast (see Figure 4.8 (b)), there was little to almost no reduction on either of the samples. Keeping these results as a benchmark, the test limits were pushed while testing the micro-pillared samples to see if the bacterial killing could occur in even shorter durations. From Figure 4.8 (c) it can be observed that both SPEEK and SPEEK-Cu micro-pillared films initially showed 4 log reduction of *E. coli* killing within 15 minutes, but yet again progressively as the films aged, they started to show reduced antimicrobial activity. Nevertheless, SPEEK and SPEEK-Cu micro-pillared film still showed 1.22 and 2.4 log reduction (~ 94% and 99.6% bacterial reduction) respectively in 15 minutes on day 15. To check if 100% killing can be achieved and also to stick with a common time period as passed in regulation by the EPA, the micro-pillared samples were exposed to 1-hour tests from day 15 onwards (see Figure 4.8 (d)).

As expected, the SPEEK-Cu micro-pillared film showed over 4 log reduction of *E. coli* and SPEEK micro-pillared film showed *E. coli* reduction ranging from 1.2 log (~94% killing) on day 15 to

0.88 log (~87% killing) on day 30. From Figure 4.8 (e) it can be observed that SPEEK micro-pillar film showed a 0.15 log reduction of yeast (~30% killing) almost consistently throughout the 1-month test duration. The SPEEK – Cu micro-pillared film showed yeast reduction ranging from over log 4 (~100% killing) on day 0 to log 0.82 (~85% killing) on day 30. This was a significant improvement over flat films. To further test its antimicrobial capabilities, the fungi *Aspergillus niger* was used to test the effects on a different type of fungi. It was observed that (see Figure 4.8 (f)) the SPEEK micro-pillared film showed log 0.22 reduction of *Aspergillus* (~40% killing) consistently throughout the 1-month test duration and the SPEEK-Cu micro-pillared film showed *Aspergillus* reduction ranging from log 0.69 (~80% killing) on day 0 to log 0.45 (~ 65% killing) on day 30. All these tests showed that micro-pillared films displayed better antimicrobial properties than flat films. Figures 4.9, 4.10 and 4.11 show visual representations of *E. coli*, yeast and *Aspergillus* growth respectively.





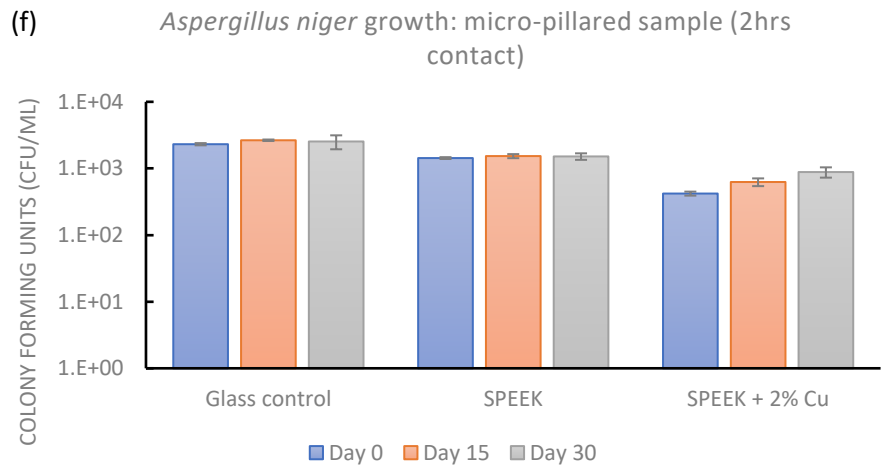


Figure 4. 8: Antimicrobial efficacy test results (a) *E. coli* growth: flat sample (1hr contact) (b) Yeast growth: flat sample (c) *E. coli* growth: micro-pillared sample (15 min contact) (d) *E. coli* growth: micro-pillared sample (1hr contact) (e) Yeast growth: micro-pillared sample (2hrs contact) (f) *Aspergillus* growth: micro-pillared sample (2hrs contact) Antimicrobial efficacy tests post sample recycling

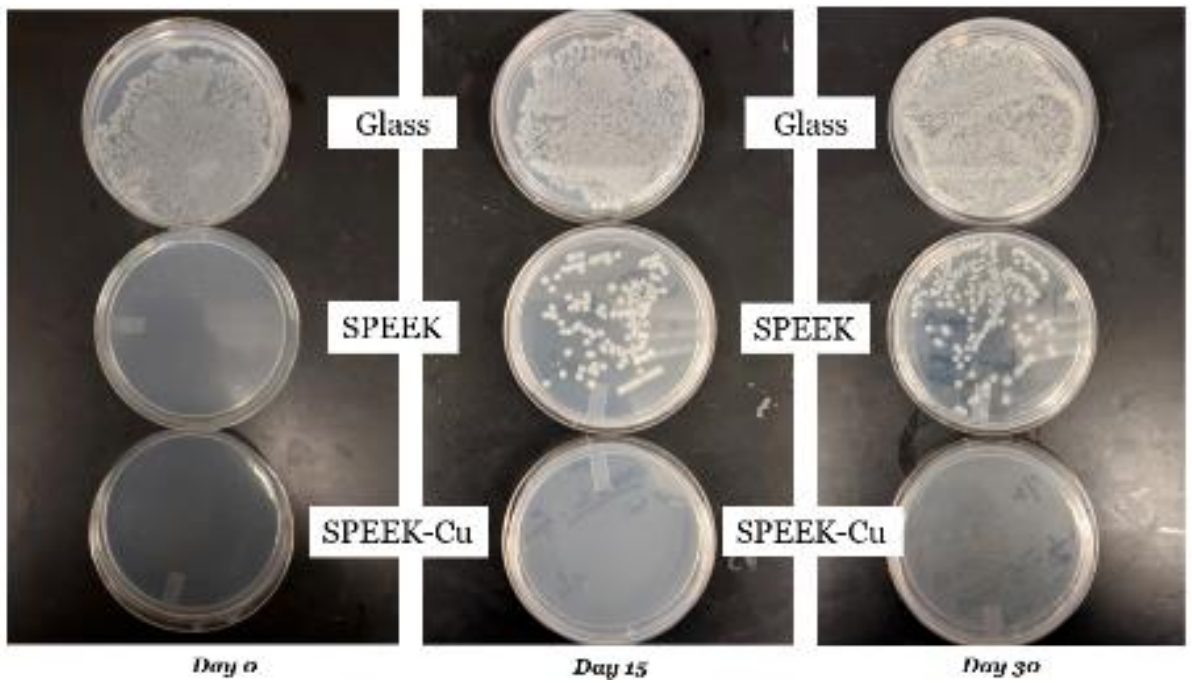


Figure 4. 9: Visual representation of *E. coli* growth with sample aging

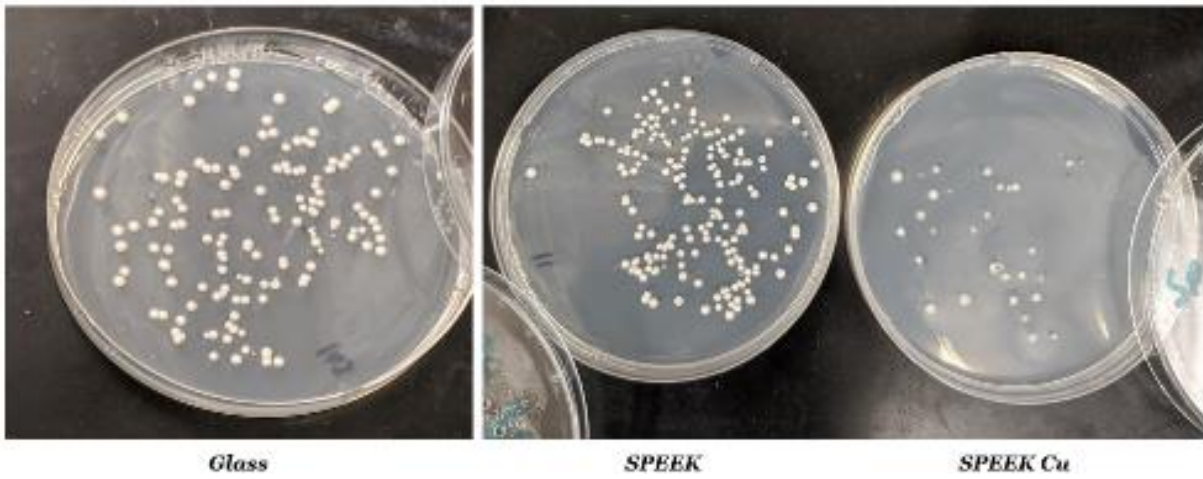


Figure 4. 10: Visual representation of yeast growth

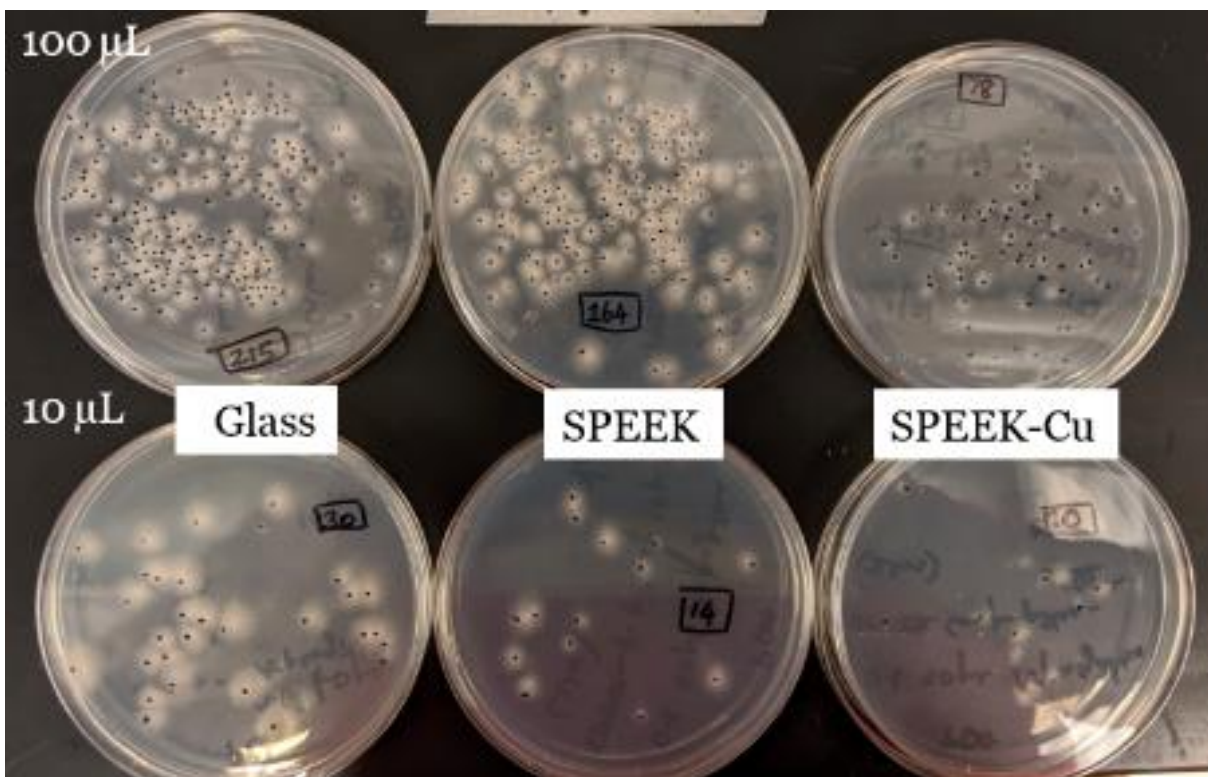
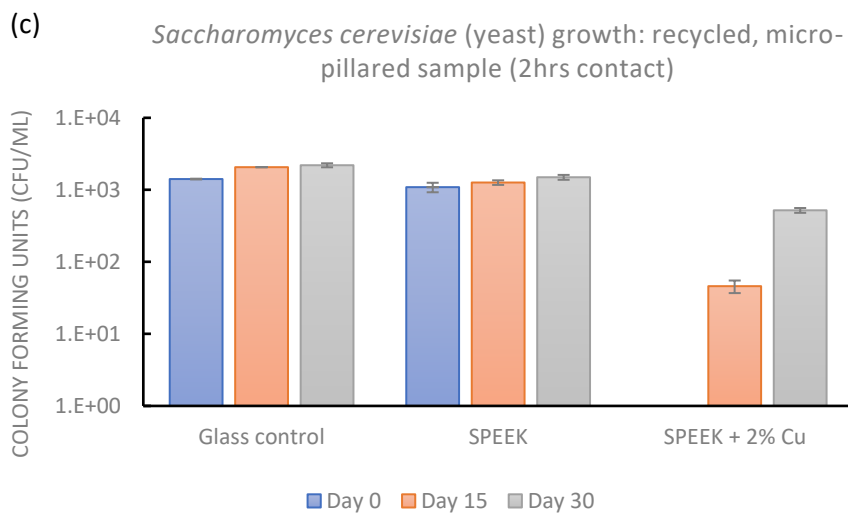
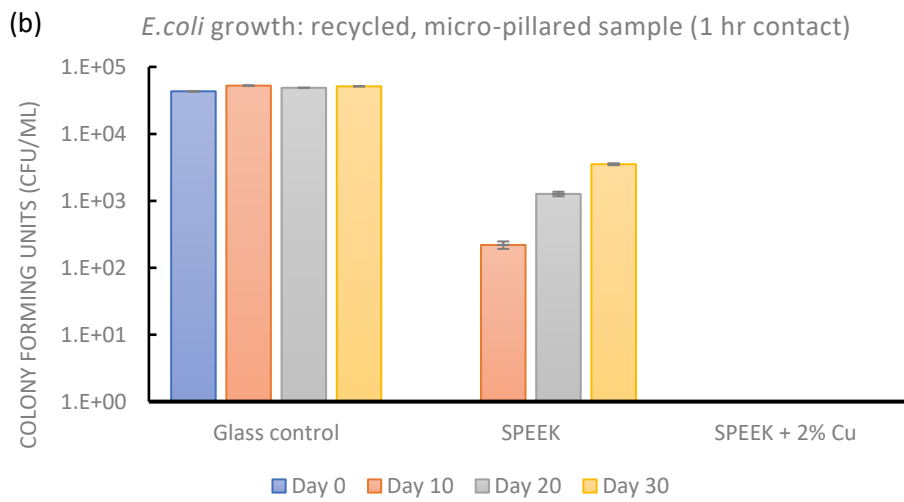
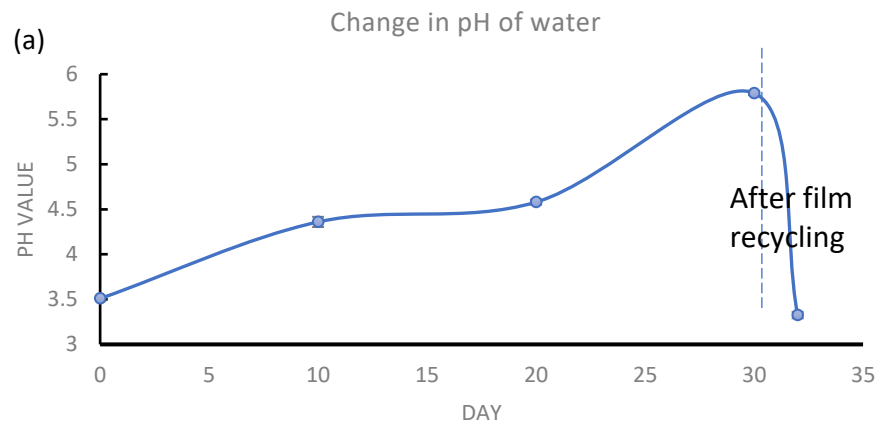


Figure 4. 11: Visual representation of Aspergillus growth

4.3.6 Antimicrobial efficacy tests post sample recycling

As observed from the results in Section 4.2.5, the antimicrobial activity of the tested samples showed a decline when the samples aged. This is due to the depletion of sulphonic acid functional groups and possible partial flaking of copper micro-particles from the surface as a result of sample handling, washing etc. The depletion of sulphonic acid functional groups was observed by a simple experiment which involved periodic pH assessment. The SPEEK – Cu film was immersed in a fresh batch of 50 mL de-ionized water everyday over a month (*this was performed with the same SPEEK – Cu sample that was being used for antimicrobial tests, in between the tests*). The water sample was collected once in 24 hours and its pH was analyzed using a pH meter. From the results in Figure 4.12 (a) it was observed that the pH of the water samples increased as the film aged, showing the reduced potential of the film to impart acidity to the solution.

To overcome this loss of activity, recycling was proposed and tested as a mechanism for renewing the material. Recycling was performed by dissolving the used films in sulphuric acid and precipitating SPEEK which was then dissolved in NMP with added copper micro-particles (for SPEEK – Cu films) and re-cast. Recycling replenishes the sulphonic acid groups restoring one of the primary killing mechanisms, thus restoring and increasing the antimicrobial properties. Once the recycled samples were produced, the same series of month-long antimicrobial tests were performed on them using *E. coli*, *Saccharomyces cerevisiae* (yeast) and *Aspergillus niger*. Significant improvements could be noted by comparing Figure 4.8 (d) with 4.12 (b) where it is clear that the same SPEEK micro-pillared samples which displayed 0.88 log reduction of *E. coli* on day 30, displayed over 4 log reduction after recycling. Similar trends were observed for SPEEK – Cu micro-pillared films with yeast and *Aspergillus* as well, as illustrated in Figure 4.12 (c) and 4.12 (d).



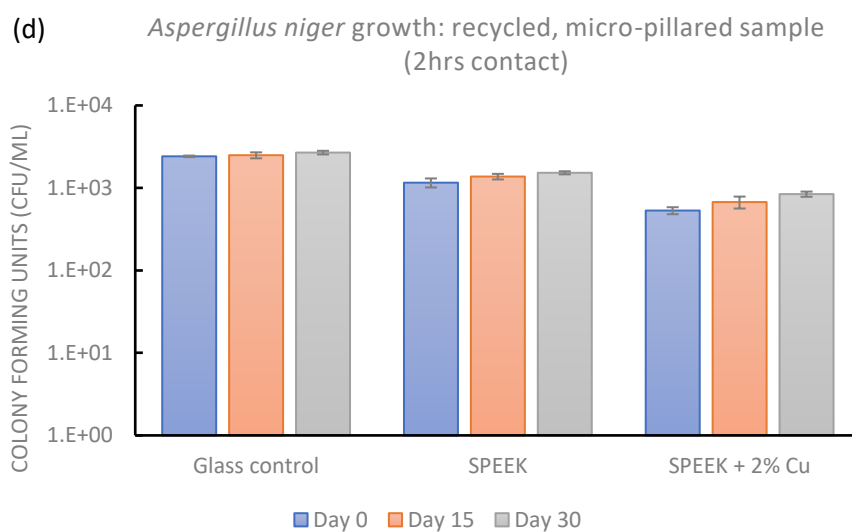


Figure 4. 12: (a) Periodic pH assessment of water samples in which the SPEEK - Cu film was immersed. Antimicrobial efficacy test results post sample recycling: (b) *E. coli* growth: recycled, micro-pillared sample (1hr contact) (c) Yeast growth: recycled, micro-pillared sample (2hrs contact) (d) *Aspergillus niger* growth: recycled, micro-pillared sample (2hrs contact)

4.4 Conclusions

From this work it can be concluded that both SPEEK and SPEEK Cu films possess antimicrobial properties, but the addition of copper microparticles significantly enhances the activity. However, over a longer term of 30 days as the films age, the micro-pillared films, especially SPEEK Cu micro-pillared film displayed over 4 log reduction of *E. coli* (>99.99% killing) within 1 hour of contact. It also showed significant fungal growth reduction for *Saccharomyces cerevisiae* (yeast) and *Aspergillus niger* with 2 hours of contact. The micro-pillared films had greater water contact angle which rendered it hydrophobic through the formation of a Cassie-Baxter state. While films containing micro-pillars had a lower tensile strength than flat ones (~35% reduction for SPEEK micro-pillared film and ~27% reduction for SPEEK - Cu micro-pillared film), they displayed improved antimicrobial properties and copper release. Furthermore, the micro-pillared samples displayed better exposure of copper micro-particles on the surface of the material via the micro-pillars. These films in general were thermally stable as they did not show

drastic weight loss by thermogravimetric analysis. Finally, the films formed can be recycled by dissolution in sulphuric acid and re-casting to form new films with replenished sulphonic acid in the polymer matrix which restores the antimicrobial properties of the film. With its excellent antimicrobial properties and recyclability, this material has promise for continued development and potential applications.

Chapter 5

Concluding Remarks and Recommendations

5.1 Conclusions

The main aim of this project was to investigate a novel antimicrobial polymer composite film with exceptional physical and chemical properties. As a precursor, an industry funded project with Aereus Technologies Inc. was undertaken, which involved evaluating the antimicrobial properties of their samples in gradual and rapid oxidizing conditions. From this industry project, it was observed that a select set of samples containing proprietary “Aereus Shield” particles withstood both gradual and rapid oxidizing conditions, maintaining their antimicrobial activity. This work was essentially quality analysis as the results provided insight to the company for product development. Based on the skills gained from this project, a well-defined project was carried out to study the effects of surface morphology on the antimicrobial properties of SPEEK – Cu composite films.

Solution casting with NMP in a glass petri-dish or a PDMS negative micro-pillar mold was used to synthesize composite films with flat and micro-pillared surface morphology. Both flat and micro-pillared films exhibited exceptional antimicrobial activity by displaying over 4 log reduction of *E. coli* (> 99.99% killing) within 1 hour of contact and significant fungal growth reduction for *Saccharomyces cerevisiae* (yeast) and *Aspergillus niger* with 2 hours of contact. While the tensile properties of the micro-pillared film was slightly lower than that of flat films, it showed better copper micro-particle exposure and release of ions, making it the generally superior morphology. The synthesized films were also thermally stable, indicating that they could operate under extreme temperatures without being degraded. With recyclability an important concern for modern products, these films are completely recyclable, as they can be dissolved back in sulphuric acid and re-cast to form new films with replenished antimicrobial properties.

5.2 Future works

For future works, the results obtained from the company project presented in Chapter 3 will help Aereus Technologies Inc. to have better understanding of their product based upon which development can be performed to improve and make their product stand out in the commercial market.

Following results from Chapter 4, further experiments could be performed to study the microscopic bacterial networks on the micro-pillars. Surface area analysis can be performed on the micro-pillared surfaces to have a detailed observation about contact area effects on antimicrobial efficacy (on both pre and post recycled samples). The effects of shear and abrasion on the micro-pillars would provide insight into the change in physical properties and antimicrobial activity of the material with extended usage. The size and size distribution of the copper micro-particles could also be varied to investigate their effects on the stability of the product and its antimicrobial efficacy. The role of copper ion's oxidation state in the antimicrobial action is also another interesting area that can be studied.

With the current COVID-19 pandemic, it has become essential to constantly disinfect surfaces and keep them clean. Improved antimicrobial touch surfaces can to an extent be employed to reduce the chance of microorganism survival on these surfaces. Because of this, there is a constant need to develop and improve antimicrobial touch surfaces, as bacteria and fungi further develop antimicrobial resistance over time. In this research, novel sulphonated poly (ether ether ketone) – copper composite films were synthesized as promising antimicrobial materials, and the effects of fabricating surface structures (micro-pillars) were investigated. With its excellent antimicrobial properties and recyclability, this material has promise for continued development and potential application. Since the presented research is novel, we believe that it will provide scientific insights for researchers in this field, after which its performance can be evaluated and compared with already existing products before putting it into practical use.

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References

- [1] A. Singh, A. Tiwari, J. Bajpai, and A. K. Bajpai, *Polymer-Based Antimicrobial Coatings as Potential Biomaterials*. Elsevier Inc., 2018.
- [2] A. Y. Peleg and D. C. Hooper, “Hospital-Acquired Infections Due to Gram-Negative Bacteria,” *N. Engl. J. Med.*, vol. 362, no. 19, pp. 1804–1813, May 2010.
- [3] S. S. Magill *et al.*, “Multistate Point-Prevalence Survey of Health Care–Associated Infections,” *N. Engl. J. Med.*, vol. 370, no. 13, pp. 1198–1208, Mar. 2014.
- [4] R. M. Klevens *et al.*, “Estimating Health Care-Associated Infections and Deaths in U.S. Hospitals, 2002,” *Public Health Rep.*, vol. 122, no. 2, pp. 160–166, Mar. 2007.
- [5] A. Peretz, A. Koiefman, E. Dinisman, D. Brodsky, and K. Labay, “Do wheelchairs spread pathogenic bacteria within hospital walls?,” *World J. Microbiol. Biotechnol.*, vol. 30, no. 2, pp. 385–387, Feb. 2014.
- [6] J. M. Bartley and R. N. Olmsted, “Reservoirs of Pathogens Causing Health Care-Associated Infections in the 21st Century: Is Renewed Attention to Inanimate Surfaces Warranted?,” *Clin. Microbiol. Newsl.*, vol. 30, no. 15, pp. 113–117, Aug. 2008.
- [7] Canada, “The Chief Public Health Officer’s Report on the State of Public Health in Canada, 2013: Infectious Disease - The Never-ending Threat,” 2013.
- [8] G. . French, J. . Otter, K. . Shannon, N. M. . Adams, D. Watling, and M. . Parks, “Tackling contamination of the hospital environment by methicillin-resistant *Staphylococcus aureus* (MRSA): a comparison between conventional terminal cleaning and hydrogen peroxide vapour decontamination,” *J. Hosp. Infect.*, vol. 57, no. 1, pp. 31–37, May 2004.
- [9] T. R. Wilkinson, “Survival of bacteria on metal surfaces.,” *Appl. Microbiol.*, vol. 14, no. 3, pp. 303–307, May 1966.

- [10] A. Kramer and O. Assadian, "Survival of microorganisms on inanimate surfaces," in *Use of Biocidal Surfaces for Reduction of Healthcare Acquired Infections*, vol. 9783319080574, Springer International Publishing, 2014, pp. 7–26.
- [11] L. Tamayo, M. Azócar, M. Kogan, A. Riveros, and M. Páez, "Copper-polymer nanocomposites: An excellent and cost-effective biocide for use on antibacterial surfaces," *Mater. Sci. Eng. C*, vol. 69, pp. 1391–1409, Dec. 2016.
- [12] H. Palza, R. Quijada, and K. Delgado, "Antimicrobial polymer composites with copper micro- and nanoparticles: Effect of particle size and polymer matrix," *J. Bioact. Compat. Polym.*, vol. 30, no. 4, pp. 366–380, 2015.
- [13] I. V. Panayotov, V. Orti, F. Cuisinier, and J. Yachouh, "Polyetheretherketone (PEEK) for medical applications," *Journal of Materials Science: Materials in Medicine*, vol. 27, no. 7. Springer New York LLC, 01-Jul-2016.
- [14] L. Wang *et al.*, "Polyetheretherketone/nano-fluorohydroxyapatite composite with antimicrobial activity and osseointegration properties," *Biomaterials*, vol. 35, no. 25, pp. 6758–6775, 2014.
- [15] H. Kakinuma *et al.*, "Antibacterial polyetheretherketone implants immobilized with silver ions based on chelate-bonding ability of inositol phosphate: Processing, material characterization, cytotoxicity, and antibacterial properties," *J. Biomed. Mater. Res. Part A*, vol. 103, no. 1, pp. 57–64, Jan. 2015.
- [16] A. Cruz-Pacheco *et al.*, "Coating of Polyetheretherketone Films with Silver Nanoparticles by a Simple Chemical Reduction Method and Their Antibacterial Activity," *Coatings*, vol. 9, no. 2, p. 91, Feb. 2019.
- [17] R. S. Brum *et al.*, "On the sulphonated PEEK for implant dentistry: Biological and physicochemical assessment," *Mater. Chem. Phys.*, vol. 223, pp. 542–547, Feb. 2019.

- [18] J. F. D. Montero *et al.*, “Biofilm behavior on sulfonated poly(ether-ether-ketone) (sPEEK),” *Mater. Sci. Eng. C*, vol. 70, pp. 456–460, 2017.
- [19] B. S. T. Peddinti, F. Scholle, M. G. Vargas, S. D. Smith, R. A. Ghiladi, and R. J. Spontak, “Inherently self-sterilizing charged multiblock polymers that kill drug-resistant microbes in minutes,” *Mater. Horizons*, 2019.
- [20] M. Vincent, R. E. Duval, P. Hartemann, and M. Engels-Deutsch, “Contact killing and antimicrobial properties of copper,” *J. Appl. Microbiol.*, vol. 124, no. 5, pp. 1032–1046, 2018.
- [21] V. K. Champagne and D. J. Helfritch, “A demonstration of the antimicrobial effectiveness of various copper surfaces,” *J. Biol. Eng.*, vol. 7, no. 1, p. 8, 2013.
- [22] O. Baghriche *et al.*, “Advantages of highly ionized pulse plasma magnetron sputtering (HIPIMS) of silver for improved E. coli inactivation,” *Thin Solid Films*, vol. 520, no. 9, pp. 3567–3573, Feb. 2012.
- [23] O. Baghriche *et al.*, “Ag-surfaces sputtered by DC and pulsed DC-magnetron sputtering effective in bacterial inactivation: Testing and characterization,” *Surf. Coatings Technol.*, vol. 206, no. 8–9, pp. 2410–2416, Jan. 2012.
- [24] S. X. Jiang, W. F. Qin, R. H. Guo, and L. Zhang, “Surface functionalization of nanostructured silver-coated polyester fabric by magnetron sputtering,” *Surf. Coatings Technol.*, vol. 204, no. 21–22, pp. 3662–3667, Aug. 2010.
- [25] M. I. Mejía *et al.*, “Magnetron-Sputtered Ag Surfaces. New Evidence for the Nature of the Ag Ions Intervening in Bacterial Inactivation,” *ACS Appl. Mater. Interfaces*, vol. 2, no. 1, pp. 230–235, Jan. 2010.
- [26] S. SANT, K. GILL, and R. BURRELL, “Nanostructure, dissolution and morphology

- characteristics of microcidal silver films deposited by magnetron sputtering☆,” *Acta Biomater.*, vol. 3, no. 3, pp. 341–350, May 2007.
- [27] J. Scholz, G. Nocke, F. Hollstein, and A. Weissbach, “Investigations on fabrics coated with precious metals using the magnetron sputter technique with regard to their anti-microbial properties,” *Surf. Coatings Technol.*, vol. 192, no. 2–3, pp. 252–256, Mar. 2005.
- [28] J. Kratochvíl, A. Kuzminova, and O. Kylián, “State-of-the-Art, and Perspectives of, Silver/Plasma Polymer Antibacterial Nanocomposites,” *Antibiotics*, vol. 7, no. 3, p. 78, 2018.
- [29] D. N. Bikiaris and K. S. Triantafyllidis, “HDPE/Cu-nanofiber nanocomposites with enhanced antibacterial and oxygen barrier properties appropriate for food packaging applications,” *Mater. Lett.*, vol. 93, pp. 1–4, Feb. 2013.
- [30] B. Xue, Y. Jiang, and G. Li, “Preparation of Cu/Dickite/LLDPE nanocomposites and synergistic effect of exfoliated dickite and nano-Cu in LLDPE matrix,” *Polym. Compos.*, vol. 34, no. 7, pp. 1061–1070, Jul. 2013.
- [31] M. Hundáková, M. Valášková, M. Samlíková, and E. Pazdziora, “Vermiculite With Ag and Cu Used as an Antibacterial Nanofiller in Polyethylene/ Vermikulit S Ag A Cu Použitý Jako Antibakteriální Nanoplivo V Polyethylenu,” *Geosci. Eng.*, vol. 60, no. 3, pp. 28–36, Oct. 2014.
- [32] Z. Komeily-Nia, M. Montazer, and M. Latifi, “Synthesis of nano copper/nylon composite using ascorbic acid and CTAB,” *Colloids Surfaces A Physicochem. Eng. Asp.*, vol. 439, pp. 167–175, Dec. 2013.
- [33] S. S. Mahapatra and N. Karak, “Hyperbranched Polyamine/Cu Nanoparticles for Epoxy Thermoset,” *J. Macromol. Sci. Part A*, vol. 46, no. 3, pp. 296–303, Jan. 2009.

- [34] L. Ploux, M. Mateescu, K. Anselme, and K. Vasilev, "Antibacterial Properties of Silver-Loaded Plasma Polymer Coatings," *J. Nanomater.*, vol. 2012, pp. 1–9, 2012.
- [35] V. Kumar, C. Jolivald, J. Pulpytel, R. Jafari, and F. Arefi-Khonsari, "Development of silver nanoparticle loaded antibacterial polymer mesh using plasma polymerization process," *J. Biomed. Mater. Res. Part A*, vol. 101A, no. 4, pp. 1121–1132, Apr. 2013.
- [36] A. Fahmy, J. Friedrich, F. Poncin-Epaillard, and D. Debarnot, "Plasma polymerized allyl alcohol/O₂ thin films embedded with silver nanoparticles," *Thin Solid Films*, vol. 616, pp. 339–347, Oct. 2016.
- [37] O. Kylián *et al.*, "Ag/C:F Antibacterial and hydrophobic nanocomposite coatings," *Funct. Mater. Lett.*, vol. 10, no. 03, p. 1750029, Jun. 2017.
- [38] S. Mathews, M. Hans, F. Mücklich, and M. Solioz, "Contact Killing of Bacteria on Copper Is Suppressed if Bacterial-Metal Contact Is Prevented and Is Induced on Iron by Copper Ions," *Appl. Environ. Microbiol.*, vol. 79, no. 8, pp. 2605–2611, Apr. 2013.
- [39] R. Jeziórska, M. Zielecka, B. Gutarowska, and Z. Żakowska, "High-Density Polyethylene Composites Filled with Nanosilica Containing Immobilized Nanosilver or Nanocopper: Thermal, Mechanical, and Bactericidal Properties and Morphology and Interphase Characterization," *Int. J. Polym. Sci.*, vol. 2014, pp. 1–13, 2014.
- [40] B. Aslam *et al.*, "Antibiotic resistance: a rundown of a global crisis," *Infection and Drug Resistance*, vol. 11. Dove Medical Press Ltd., pp. 1645–1658, 2018.
- [41] B. Li and T. J. Webster, "Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections," *J. Orthop. Res.*, vol. 36, no. 1, pp. 22–32, Jan. 2018.
- [42] L. Rojo, J. M. Barcenilla, B. Vázquez, R. González, and J. San Román, "Intrinsically

- antibacterial materials based on polymeric derivatives of eugenol for biomedical applications,” *Biomacromolecules*, vol. 9, no. 9, pp. 2530–2535, 2008.
- [43] D. Campoccia, L. Montanaro, and C. R. Arciola, “A review of the biomaterials technologies for infection-resistant surfaces,” *Biomaterials*, vol. 34, no. 34, pp. 8533–8554, 2013.
- [44] C. J. Wilson, R. E. Clegg, D. I. Leavesley, and M. J. Percy, “Mediation of Biomaterial–Cell Interactions by Adsorbed Proteins: A Review,” *Tissue Eng.*, vol. 11, no. 1–2, pp. 1–18, 2005.
- [45] I. S. Yeo, H. Y. Kim, K. S. Lim, and J. S. Han, “Implant surface factors and bacterial adhesion: A review of the literature,” *Int. J. Artif. Organs*, vol. 35, no. 10, pp. 762–772, 2012.
- [46] L. Bacakova, E. Filova, M. Parizek, T. Ruml, and V. Svorcik, “Modulation of cell adhesion, proliferation and differentiation on materials designed for body implants,” *Biotechnol. Adv.*, vol. 29, no. 6, pp. 739–767, 2011.
- [47] L. Badihi Hauslich, M. N. Sela, D. Steinberg, G. Rosen, and D. Kohavi, “The adhesion of oral bacteria to modified titanium surfaces: Role of plasma proteins and electrostatic forces,” *Clin. Oral Implants Res.*, vol. 24, no. A100, pp. 49–56, 2013.
- [48] H. Dale *et al.*, “Infection after primary hip arthroplasty: A comparison of 3 Norwegian health registers,” *Acta Orthop.*, vol. 82, no. 6, pp. 646–654, 2011.
- [49] K. Anselme, P. Davidson, A. M. Popa, M. Giazzon, M. Liley, and L. Ploux, “The interaction of cells and bacteria with surfaces structured at the nanometre scale,” *Acta Biomaterialia*, vol. 6, no. 10. Acta Biomater, pp. 3824–3846, Oct-2010.
- [50] N. Mitik-Dineva *et al.*, “*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* Attachment Patterns on Glass Surfaces with Nanoscale Roughness,” *Curr.*

- Microbiol.*, vol. 58, no. 3, pp. 268–273, Mar. 2009.
- [51] S. D. Puckett, E. Taylor, T. Raimondo, and T. J. Webster, “The relationship between the nanostructure of titanium surfaces and bacterial attachment,” *Biomaterials*, vol. 31, no. 4, pp. 706–713, Feb. 2010.
- [52] N. Mitik-Dineva, J. Wang, R. C. Mocanasi, P. R. Stoddart, R. J. Crawford, and E. P. Ivanova, “Impact of nano-topography on bacterial attachment,” *Biotechnol. J.*, vol. 3, no. 4, pp. 536–544, Apr. 2008.
- [53] E. Taylor and T. J. Webster, “Reducing infections through nanotechnology and nanoparticles,” *Int. J. Nanomedicine*, vol. 6, pp. 1463–1473, 2011.
- [54] L. S. Nair and C. T. Laurencin, “Nanofibers and nanoparticles for orthopaedic surgery applications,” *J. Bone Jt. Surg. - Ser. A*, vol. 90, no. SUPPL. 1, pp. 128–131, 2008.
- [55] D. Campoccia *et al.*, “Study of Staphylococcus aureus adhesion a novel nanostructured surface by chemiluminometry,” *Int. J. Artif. Organs*, vol. 29, no. 6, pp. 622–629, 2006.
- [56] H. Lin, L. Ding, W. Deng, and X. Wang, “Coating of Medical-Grade PVC Material with ZnO for Antibacterial Application,” *Adv. Chem. ...*, vol. 29, no. 3, pp. 423–32, 2013.
- [57] C. Chagnot, M. A. Zorgani, T. Astruc, and M. Desvaux, “Proteinaceous determinants of surface colonization in bacteria: bacterial adhesion and biofilm formation from a protein secretion perspective,” *Front. Microbiol.*, vol. 4, no. October, pp. 1–26, 2013.
- [58] C. R. Jenney and J. M. Anderson, “Adsorbed serum proteins responsible for surface dependent human macrophage behavior,” *J. Biomed. Mater. Res.*, vol. 49, no. 4, pp. 435–447, 2000.
- [59] P. Roach, D. Eglin, K. Rohde, and C. C. Perry, “Modern biomaterials: A review - Bulk properties and implications of surface modifications,” *J. Mater. Sci. Mater. Med.*, vol. 18,

- no. 7, pp. 1263–1277, 2007.
- [60] G. Gosheger *et al.*, “Silver-coated megaendoprostheses in a rabbit model - An analysis of the infection rate and toxicological side effects,” *Biomaterials*, vol. 25, no. 24, pp. 5547–5556, 2004.
- [61] R. O. Darouiche, R. Meade, M. Mansouri, and I. I. Raad, “In vivo efficacy of antimicrobial-coated fabric from prosthetic heart valve sewing rings,” *J.Heart Valve Dis.*, 1998.
- [62] R. Kumar and H. Münstedt, “Silver ion release from antimicrobial polyamide/silver composites,” *Biomaterials*, vol. 26, no. 14, pp. 2081–2088, 2005.
- [63] J. C. Tiller, C.-J. Liao, K. Lewis, and A. M. Klibanov, “Designing surfaces that kill bacteria on contact,” *Proc. Natl. Acad. Sci.*, vol. 98, no. 11, pp. 5981–5985, 2001.
- [64] K. Hegstad, S. Langsrud, B. T. Lunestad, A. A. Scheie, M. Sunde, and S. P. Yazdankhah, “Does the wide use of quaternary ammonium compounds enhance the selection and spread of antimicrobial resistance and thus threaten our health?,” *Microbial Drug Resistance*, vol. 16, no. 2. Mary Ann Liebert Inc., pp. 91–104, 01-Jun-2010.
- [65] S. Takenaka, T. Tonoki, K. Taira, S. Murakami, and K. Aoki, “Adaptation of *Pseudomonas* sp. strain 7-6 to quaternary ammonium compounds and their degradation via dual pathways,” *Appl. Environ. Microbiol.*, vol. 73, no. 6, pp. 1799–1802, Mar. 2007.
- [66] G. Franci *et al.*, “Silver nanoparticles as potential antibacterial agents,” *Molecules*, vol. 20, no. 5. MDPI AG, pp. 8856–8874, 2015.
- [67] N. Verma and N. Kumar, “Synthesis and Biomedical Applications of Copper Oxide Nanoparticles: An Expanding Horizon,” *ACS Biomater. Sci. Eng.*, vol. 5, no. 3, pp. 1170–1188, Mar. 2019.
- [68] A. C. Burduşel, O. Gherasim, A. M. Grumezescu, L. Mogoantă, A. Ficai, and E.

- Andronesu, “Biomedical applications of silver nanoparticles: An up-to-date overview,” *Nanomaterials*, vol. 8, no. 9. MDPI AG, 01-Sep-2018.
- [69] M. G. Gordienko *et al.*, “Antimicrobial activity of silver salt and silver nanoparticles in different forms against microorganisms of different taxonomic groups,” *J. Hazard. Mater.*, vol. 378, p. 120754, Oct. 2019.
- [70] J. L. Clement and P. S. Jarrett, “Antibacterial Silver,” *Met. Based. Drugs*, vol. 1, no. 5–6, pp. 467–482, 1994.
- [71] S. J. Berners-Price, R. K. Johnson, A. J. Giovenella, L. F. Faucette, C. K. Mirabelli, and P. J. Sadler, “Antimicrobial and anticancer activity of tetrahedral, chelated, diphosphine silver(I) complexes: Comparison with copper and gold,” *J. Inorg. Biochem.*, vol. 33, no. 4, pp. 285–295, Aug. 1988.
- [72] G. Gosheger *et al.*, “Silver-coated megaendoprostheses in a rabbit model - An analysis of the infection rate and toxicological side effects,” *Biomaterials*, vol. 25, no. 24, pp. 5547–5556, Nov. 2004.
- [73] D. Lee, R. E. Cohen, and M. F. Rubner, “Antibacterial properties of Ag nanoparticle loaded multilayers and formation of magnetically directed antibacterial microparticles,” *Langmuir*, vol. 21, no. 21, pp. 9651–9659, Oct. 2005.
- [74] I. A. Rojas, J. B. Slunt, and D. W. Grainger, “Polyurethane coatings release bioactive antibodies to reduce bacterial adhesion,” *J. Control. Release*, vol. 63, no. 1–2, pp. 175–189, Jan. 2000.
- [75] W. H. Song, S. R. Hyun, and S. H. Hong, “Antibacterial properties of Ag (or Pt)-containing calcium phosphate coatings formed by micro-arc oxidation,” *J. Biomed. Mater. Res. - Part A*, vol. 88, no. 1, pp. 246–254, Jan. 2009.

- [76] H. T. Michels, C. W. Keevil, C. D. Salgado, and M. G. Schmidt, "From laboratory research to a clinical trial: copper alloy surfaces kill bacteria and reduce hospital-acquired infections," *Heal. Environ. Res. Des. J.*, vol. 9, no. 1, pp. 64–79, Jan. 2015.
- [77] W. Moran *et al.*, "Risk Mitigation of Hospital Acquired Infections Through the Use of Antimicrobial Copper Surfaces."
- [78] N. S. McIntyre and M. G. Cook, "X-Ray Photoelectron Studies on Some Oxides and Hydroxides of Cobalt, Nickel, and Copper," *Anal. Chem.*, vol. 47, no. 13, pp. 2208–2213, Nov. 1975.
- [79] C. E. Santo, D. Quaranta, and G. Grass, "Antimicrobial metallic copper surfaces kill *Staphylococcus haemolyticus* via membrane damage," *Microbiologyopen*, vol. 1, no. 1, pp. 46–52, Mar. 2012.
- [80] J. A. Lemire, J. J. Harrison, and R. J. Turner, "Antimicrobial activity of metals: Mechanisms, molecular targets and applications," *Nature Reviews Microbiology*, vol. 11, no. 6. *Nat Rev Microbiol*, pp. 371–384, Jun-2013.
- [81] L. Macomber, C. Rensing, and J. A. Imlay, "Intracellular copper does not catalyze the formation of oxidative DNA damage in *Escherichia coli*," *J. Bacteriol.*, vol. 189, no. 5, pp. 1616–1626, Mar. 2007.
- [82] R. Kumar and H. Müntstedt, "Silver ion release from antimicrobial polyamide/silver composites," *Biomaterials*, vol. 26, no. 14, pp. 2081–2088, May 2005.
- [83] R. J. Cornell and L. G. Donaruma, "2-Methacryloxytropones. Intermediates for the Synthesis of Biologically Active Polymers," *J. Med. Chem.*, vol. 8, no. 3, pp. 388–390, May 1965.
- [84] L. G. Donaruma and J. Razzano, "Synthetic Biologically Active Polymers. III. A

- Sulfapyridine-Formaldehyde Copolymer,” *J. Med. Chem.*, vol. 9, no. 2, pp. 258–259, Mar. 1966.
- [85] J. R. Dombroski, L. G. Donaruma, and J. Razzano, “Synthetic Biologically Active Polymers. IV. N¹-Acylsulfanilamide-Formaldehyde Copolymers,” *J. Med. Chem.*, vol. 10, no. 5, pp. 963–964, Sep. 1967.
- [86] J. R. Dombroski, J. Razzano, and L. G. Donaruma, “Synthetic Biologically Active Polymers. 9. Comparison of Antimalarial Activity in Copolymers Containing Common Sulfonamide Monomers but Different Comonomers,” *Journal of Medicinal Chemistry*, vol. 14, no. 10. *J Med Chem*, p. 993, 01-Oct-1971.
- [87] L. G. Donaruma and J. R. Dombroski, “Synthetic biologically active polymers. 8. Antibacterial activity of some sulfonamide-dimethylolurea copolymers,” *J. Med. Chem.*, vol. 14, no. 5, pp. 460–461, May 1971.
- [88] C. E. Malion, B. H. Danree, and X. B. L. Pascaud, “New Thiocarboxamides Derivatives with Specific Gastric Antisecretory Properties,” *J. Med. Chem.*, vol. 14, no. 3, p. 244, Mar. 1971.
- [89] E. R. Kenawy, S. D. Worley, and R. Broughton, “The chemistry and applications of antimicrobial polymers: A state-of-the-art review,” *Biomacromolecules*, vol. 8, no. 5. *Biomacromolecules*, pp. 1359–1384, May-2007.
- [90] G. J. Gabriel, A. Som, A. E. Madkour, T. Eren, and G. N. Tew, “Infectious disease: Connecting innate immunity to biocidal polymers,” *Materials Science and Engineering R: Reports*, vol. 57, no. 1–6. *Mater Sci Eng R Rep*, pp. 28–64, 01-Aug-2007.
- [91] F. J. Xu, K. G. Neoh, and E. T. Kang, “Bioactive surfaces and biomaterials via atom transfer radical polymerization,” *Progress in Polymer Science (Oxford)*, vol. 34, no. 8. Pergamon, pp. 719–761, 01-Aug-2009.

- [92] L. Timofeeva and N. Kleshcheva, “Antimicrobial polymers: Mechanism of action, factors of activity, and applications,” *Applied Microbiology and Biotechnology*, vol. 89, no. 3. Appl Microbiol Biotechnol, pp. 475–492, Feb-2011.
- [93] F. Siedenbiedel and J. C. Tiller, “Antimicrobial Polymers in Solution and on Surfaces: Overview and Functional Principles,” *Polymers (Basel)*., vol. 4, no. 1, pp. 46–71, Jan. 2012.
- [94] A. Muñoz-Bonilla and M. Fernández-García, “Polymeric materials with antimicrobial activity,” *Progress in Polymer Science (Oxford)*, vol. 37, no. 2. Elsevier Ltd, pp. 281–339, 01-Feb-2012.
- [95] B. Alcock and T. Peijs, “Technology and Development of Self-Reinforced Polymer Composites BT - Polymer Composites – Polyolefin Fractionation – Polymeric Peptidomimetics – Collagens,” in *Advances in Polymer Science*, vol. 251, A. Abe, H.-H. Kausch, M. Möller, and H. Pasch, Eds. Springer Berlin Heidelberg, 2013, pp. 1–76.
- [96] K. Kuroda and G. A. Caputo, “Antimicrobial polymers as synthetic mimics of host-defense peptides,” *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, vol. 5, no. 1. Wiley Interdiscip Rev Nanomed Nanobiotechnol, pp. 49–66, Jan-2013.
- [97] A. Muñoz-Bonilla, M. Cerrada, and M. Fernández-García, Eds., *Polymeric Materials with Antimicrobial Activity*. Cambridge: Royal Society of Chemistry, 2013.
- [98] W.-S. Moon, J. Chul Kim, K.-H. Chung, E.-S. Park, M.-N. Kim, and J.-S. Yoon, “Antimicrobial activity of a monomer and its polymer based on quinolone,” *J. Appl. Polym. Sci.*, vol. 90, no. 7, pp. 1797–1801, Nov. 2003.
- [99] A. K. Marr, W. J. Gooderham, and R. E. Hancock, “Antibacterial peptides for therapeutic use: obstacles and realistic outlook,” *Current Opinion in Pharmacology*, vol. 6, no. 5. Curr Opin Pharmacol, pp. 468–472, Oct-2006.

- [100] Y. Zhang, J. Jiang, and Y. Chen, "Synthesis and antimicrobial activity of polymeric guanidine and biguanidine salts."
- [101] M. B. Harney, R. R. Pant, P. A. Fulmer, and J. H. Wynne, "Surface self-concentrating amphiphilic quaternary ammonium biocides as coating additives," *ACS Appl. Mater. Interfaces*, vol. 1, no. 1, pp. 39–41, Jan. 2009.
- [102] G. Sauvet, W. Fortuniak, K. Kazmierski, and J. Chojnowski, "Amphiphilic block and statistical siloxane copolymers with antimicrobial activity," *J. Polym. Sci. Part A Polym. Chem.*, vol. 41, no. 19, pp. 2939–2948, Oct. 2003.
- [103] B. Dizman, M. O. Elasri, and L. J. Mathias, "Synthesis and characterization of antibacterial and temperature responsive methacrylamide polymers," *Macromolecules*, vol. 39, no. 17, pp. 5738–5746, Aug. 2006.
- [104] E. S. Park, H. S. Kim, M. N. Kim, and J. S. Yoon, "Antibacterial activities of polystyrene-block-poly(4-vinyl pyridine) and poly(styrene-random-4-vinyl pyridine)," *Eur. Polym. J.*, vol. 40, no. 12, pp. 2819–2822, Dec. 2004.
- [105] E. R. Kenawy, F. I. Abdel-Hay, A. E. R. R. El-Shanshoury, and M. H. El-Newehy, "Biologically active polymers. V. Synthesis and antimicrobial activity of modified poly(glycidyl methacrylate-co-2-hydroxyethyl methacrylate) derivatives with quaternary ammonium and phosphonium salts," *J. Polym. Sci. Part A Polym. Chem.*, vol. 40, no. 14, pp. 2384–2393, Jul. 2002.
- [106] J. Bozja, J. Sherrill, S. Michielsen, and I. Stojiljkovic, "Porphyrin-based, light-activated antimicrobial materials," *J. Polym. Sci. Part A Polym. Chem.*, vol. 41, no. 15, pp. 2297–2303, Aug. 2003.
- [107] P. Appendini and J. H. Hotchkiss, "Surface modification of poly(styrene) by the attachment of an antimicrobial peptide," *J. Appl. Polym. Sci.*, vol. 81, no. 3, pp. 609–616, Jul. 2001.

- [108] N. D. Stebbins, M. A. Ouimet, and K. E. Uhrich, "Antibiotic-containing polymers for localized, sustained drug delivery," *Advanced Drug Delivery Reviews*, vol. 78. Elsevier, pp. 77–87, 30-Nov-2014.
- [109] M. Szkudlarek, E. Heine, H. Keul, U. Beginn, and M. Möller, "Synthesis, characterization, and antimicrobial properties of peptides mimicking copolymers of maleic anhydride and 4-methyl-1-pentene," *Int. J. Mol. Sci.*, vol. 19, no. 9, Sep. 2018.
- [110] E.-S. Park, H. K. Kim, J. H. Shim, M.-N. Kim, and J.-S. Yoon, "Synthesis and properties of polymeric biocides based on poly(ethylene-co-vinyl alcohol)," *J. Appl. Polym. Sci.*, vol. 93, no. 2, pp. 765–770, Jul. 2004.
- [111] C. M. Dvoracek, G. Sukhonosova, M. J. Benedikt, and J. C. Grunlan, "Antimicrobial behavior of polyelectrolyte-surfactant thin film assemblies," *Langmuir*, vol. 25, no. 17, pp. 10322–10328, Sep. 2009.
- [112] J. C. Grunlan, J. K. Choi, and A. Lin, "Antimicrobial behavior of polyelectrolyte multilayer films containing cetrimide and silver," *Biomacromolecules*, vol. 6, no. 2, pp. 1149–1153, Mar. 2005.
- [113] K. H. Hong and G. Sun, "Photoinduced antimicrobial polymer blends with benzophenone as a functional additive," *J. Appl. Polym. Sci.*, vol. 112, no. 4, pp. 2019–2026, May 2009.
- [114] J. Ji and W. Zhang, "Bacterial behaviors on polymer surfaces with organic and inorganic antimicrobial compounds," *J. Biomed. Mater. Res. - Part A*, vol. 88, no. 2, pp. 448–453, Feb. 2009.
- [115] N. Meng, N. L. Zhou, S. Q. Zhang, and J. Shen, "Synthesis and antimicrobial activities of polymer/montmorillonite-chlorhexidine acetate nanocomposite films," *Appl. Clay Sci.*, vol. 42, no. 3–4, pp. 667–670, Jan. 2009.

- [116] H. Palza *et al.*, “Toward Tailor-Made Biocide Materials Based on Poly(propylene)/Copper Nanoparticles,” *Macromol. Rapid Commun.*, vol. 31, no. 6, pp. 563–567, Mar. 2010.
- [117] H. Jiang, S. Manolache, A. C. L. Wong, and F. S. Denes, “Plasma-enhanced deposition of silver nanoparticles onto polymer and metal surfaces for the generation of antimicrobial characteristics,” *J. Appl. Polym. Sci.*, vol. 93, no. 3, pp. 1411–1422, Aug. 2004.
- [118] H. Kong, J. Song, and J. Jang, “One-Step Preparation of Antimicrobial Polyrhodanine Nanotubes with Silver Nanoparticles,” *Macromol. Rapid Commun.*, vol. 30, no. 15, pp. 1350–1355, Aug. 2009.
- [119] V. K. Sharma, R. A. Yngard, and Y. Lin, “Silver nanoparticles: Green synthesis and their antimicrobial activities,” *Advances in Colloid and Interface Science*, vol. 145, no. 1–2, pp. 83–96, 30-Jan-2009.
- [120] H. Palza and R. Quijada, “Antimicrobial polymer composites with copper micro- and nanoparticles : Effect of particle size and polymer matrix,” 2015.
- [121] H. Palza *et al.*, “Toward Tailor-Made Biocide Materials Based on Poly(propylene)/Copper Nanoparticles,” *Macromol. Rapid Commun.*, vol. 31, no. 6, pp. 563–567, Mar. 2010.
- [122] D. N. Bikiaris and K. S. Triantafyllidis, “HDPE/Cu-nanofiber nanocomposites with enhanced antibacterial and oxygen barrier properties appropriate for food packaging applications,” *Mater. Lett.*, vol. 93, pp. 1–4, Feb. 2013.
- [123] Z. Komeily-Nia, M. Montazer, and M. Latifi, “Synthesis of nano copper/nylon composite using ascorbic acid and CTAB,” *Colloids Surfaces A Physicochem. Eng. Asp.*, vol. 439, pp. 167–175, Dec. 2013.
- [124] S. S. Mahapatra and N. Karak, “Hyperbranched polyamine/Cu nanoparticles for epoxy thermoset,” *J. Macromol. Sci. Part A Pure Appl. Chem.*, vol. 46, no. 3, pp. 296–303, Mar.

- 2009.
- [125] V. V. A. Thampi, S. Thanka Rajan, K. Anupriya, and B. Subramanian, “Functionalization of fabrics with PANI/CuO nanoparticles by precipitation route for anti-bacterial applications,” *J. Nanoparticle Res.*, vol. 17, no. 1, pp. 1–12, Jan. 2015.
- [126] D. Longano *et al.*, “Analytical characterization of laser-generated copper nanoparticles for antibacterial composite food packaging,” in *Analytical and Bioanalytical Chemistry*, 2012, vol. 403, no. 4, pp. 1179–1186.
- [127] T. Zhong, G. S. Oporto, J. Jaczynski, and C. Jiang, “Nanofibrillated cellulose and copper nanoparticles embedded in polyvinyl alcohol films for antimicrobial applications,” *Biomed Res. Int.*, vol. 2015, 2015.
- [128] N. Cioffi *et al.*, “Synthesis, analytical characterization and bioactivity of Ag and Cu nanoparticles embedded in poly-vinyl-methyl-ketone films,” *Anal. Bioanal. Chem.*, vol. 382, no. 8, pp. 1912–1918, Aug. 2005.
- [129] C. I. Nr, “Technical information and applications for PEEK fabrics,” no. 2.
- [130] T. Shen, Y. Liu, Y. Zhu, D.-Q. Yang, and E. Sacher, “Improved adhesion of Ag NPs to the polyethylene terephthalate surface via atmospheric plasma treatment and surface functionalization,” *Appl. Surf. Sci.*, vol. 411, pp. 411–418, Jul. 2017.
- [131] H. Jiang, S. Manolache, A. C. L. Wong, and F. S. Denes, “Plasma-enhanced deposition of silver nanoparticles onto polymer and metal surfaces for the generation of antimicrobial characteristics,” *J. Appl. Polym. Sci.*, vol. 93, no. 3, pp. 1411–1422, Aug. 2004.
- [132] N. A. Ibrahim, B. M. Eid, and M. S. Abdel-Aziz, “Effect of plasma superficial treatments on antibacterial functionalization and coloration of cellulosic fabrics,” *Appl. Surf. Sci.*, vol. 392, pp. 1126–1133, Jan. 2017.

- [133] C. Charton and M. Fahland, "Growth of Ag films on PET deposited by magnetron sputtering," *Vacuum*, vol. 68, no. 1, pp. 65–73, Oct. 2002.
- [134] P. Grammatikopoulos, S. Steinhauer, J. Vernieres, V. Singh, and M. Sowwan, "Nanoparticle design by gas-phase synthesis," *Adv. Phys. X*, vol. 1, no. 1, pp. 81–100, Jan. 2016.
- [135] M. Walkowicz *et al.*, "Impact of oxidation of copper and its alloys in laboratory-simulated conditions on their antimicrobial efficiency," *Corros. Sci.*, vol. 140, pp. 321–332, Aug. 2018.
- [136] M. Hans, A. Erbe, S. Mathews, Y. Chen, M. Solioz, and F. Mücklich, "Role of copper oxides in contact killing of bacteria," *Langmuir*, vol. 29, no. 52, pp. 16160–16166, Dec. 2013.
- [137] N. S. Water, "Reparation of Seawater Media for," pp. 11–13, 1993.
- [138] C. Callewaert, B. Buysschaert, E. Vossen, V. Fievez, T. Van de Wiele, and N. Boon, "Artificial sweat composition to grow and sustain a mixed human axillary microbiome," *J. Microbiol. Methods*, vol. 103, pp. 6–8, 2014.
- [139] "Updated Draft Protocol for the Evaluation of Bactericidal Activity of Hard, Non-porous Copper Containing Surface Products," *EPA*, 11-May-2017. [Online]. Available: <https://www.epa.gov/pesticide-registration/updated-draft-protocol-evaluation-bactericidal-activity-hard-non-porous>. [Accessed: 25-Feb-2020]
- [140] M. Sanjay Krishna *et al.*, "Polymer-iron tungstate-reduced graphene oxide nanocomposites for microwave absorption," *IOP Conf. Ser. Mater. Sci. Eng.*, vol. 577, no. 1, 2019.
- [141] J. C. Marrero, A. de S. Gomes, W. S. Hui, J. C. Dutra Filho, and V. S. de Oliveira, "Sulfonation degree effect on ion-conducting SPEEK-titanium oxide membranes properties," *Polímeros*, 2017.
- [142] L. Li, J. Zhang, and Y. Wang, "Sulfonated poly(ether ether ketone) membranes for direct

- methanol fuel cell,” *J. Memb. Sci.*, vol. 226, no. 1–2, pp. 159–167, Dec. 2003.
- [143] W. M. Sigmund and S.-H. Hsu, “Cassie–Baxter Model,” in *Encyclopedia of Membranes*, Springer Berlin Heidelberg, 2016, pp. 310–311.
- [144] Z. Jahed *et al.*, “Bacterial Networks on Hydrophobic Micropillars,” *ACS Nano*, vol. 11, no. 1, pp. 675–683, 2017.
- [145] S. Sonpingkam and D. Pattavarakorn, “Mechanical Properties of Sulfonated Poly (Ether Ether Ketone) Nanocomposite Membranes,” *Int. J. Chem. Eng. Appl.*, 2014.
- [146] D. Gupta and V. Choudhary, “Studies on novel heat treated sulfonated poly(ether ether ketone) [SPEEK]/diol membranes for fuel cell applications,” *Int. J. Hydrogen Energy*, vol. 36, no. 14, pp. 8525–8535, Jul. 2011.
- [147] S. Kaliaguine and M. Bousmina, “Tensile Mechanical Properties of Sulfonated Poly (Ether Ether Ketone) (SPEEK) and BPO 4 / SPEEK Membranes,” no. December 2004, 2005.