STUDIES OF ANTIMICROBIAL AGENT (AA) FILLED WITH POLYPROPYLENE (PP) FOR ANTIMICROBIAL APPLICATION

by

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I declare that the contents presented in this thesis are my own work which was done at University Science Malaysia unless stated otherwise. The thesis has not been previously submitted for any other degree.

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LIST OF ABBREVATIONS

PP	Polypropylene
AA	Antimicrobial agent
Cu	Copper
CuO	Copper oxide
TiO_2	Titanium dioxide
Ag	Silver
$AgNO_3$	Silver nitrate
ZnO	Zinc oxide
SEM	Scanning Electron Microscopy
XRD	X-ray Diffraction
DSC	Differential scanning calorimeter
C	Colour
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
E.coli	Escherichia coli
CP	Cooled under pressure of 1000psi by flushing the press with cold water
SC	Cooled to room temperature by slowly cooling overnight
QCW	Quenching in 10°C of cold water
QWW	Quenching in 30°C of warm water
ROS	Reactive oxygen species
CuP	Copper metal
CuOP	Copper oxide nanoparticles

DNA	Deoxyribonucleic acid
S. aureus	Staphylococcus aureus
B.substilis	Bacillus subtilis
UV	Ultraviolet

KAJIAN MENGENAI AGEN ANTIMIKROB (AA) DIISI DENGAN POLIPROPILENA (PP) UNTUK APLIKASI ANTIMIKROB

ABSTRAK

Jangkitan mikrob terkenal sebagai ancaman global terhadap kesihatan manusia dalam bidang bioperubatan. Jangkitan yang disebabkan oleh mikrob di hospital berkaitan dengan penggunaan peralatan perubatan terutamanya kateter. Dalam kajian ini, polimer matriks komposit dihasilkan dengan menggabungkan zarah CuO dan TiO_2 ke dalam polipropilena melalui proses pencampuran dengan Haake internal mixer pada 185°C untuk 10 minit diikuti dengan proses acuan pemampatan. Parameter yang digunakan termasuk pelbagai komposisi PP, CuO, TiO_2, Ag, $AgNO_3$ dan ZnO serta suhu proses penyejukan pada QCW (10°C), QWW (30°C), CP and SC. Pencirian yang digunakan termasuk mikroskop optik, SEM, XRD, DSC dan ujian antimikrob (kaedah terbenam dan pengiraan koloni). Terdapat tiga peringkat yang terlibat seperti kesan menggunakan pelbagai jenis AA dibenamkan dalam PP matriks, kesan campuran dua atau tiga pelbagai jenis AA dan agen warna dibenamkan dalam PP matriks serta kesan pelbagai keadaan penyejukan. Pada peringkat 1, CuO/PP dan TiO_2/PP menunjukkan penyebaran zarah yang baik dan ujian antimikrob yang baik yang kemudian digunakan dalam peringkat seterusnya. PP/CuO/TiO_2/C telah dipilih sebagai sampel terbaik disebabkan oleh taburan AA dalam PP matrix yang baik, kurang tahap penghabluran dan ketahanan antimikrob yang baik. Kaedah pencelupan dalam air suam membuatkan sampel PP/CuO/TiO_2/C lebih amorfus dan meningkatkan keberkesanan antimikrob. Kesimpulannya, PP/CuO/TiO_2/C mempunyai potensi yang tinggi untuk mengurangkan jangkitan yang disebabkan oleh mikrob. Ia juga boleh digunakan untuk menggantikan kateter yang pada masa kini sedang digunakan dalam bidang bioperubatan.

STUDIES OF ANTIMICROBIAL AGENT (AA) FILLED WITH POLYPROPYLENE (PP) FOR ANTIMICROBIAL APPLICATION

ABSTRACT

Microbial infection is well recognized as a global threat to human health in biomedical field. Infections caused by microbes in hospitals are associated with usage of medical equipment and devices especially catheter. In this study, polymer matrix composite materials was produced by incorporated CuO and TiO_2 particles into polypropylene through mixing process in Haake internal mixer at 185°C for 10 minutes followed by compression moulding process. Parameters used included variety composition of PP, CuO, TiO_2, Ag, $AgNO_3$ and ZnO and temperature of cooling process at QCW (10°C), QWW (30°C), CP and SC. Characterizations used included optical microscope, SEM, XRD, DSC and antimicrobial testing (embedded method and colony count). There were three stages involved like effect of using different types of AA embedded in PP matrix, effect of mixture of two to three different types of AA and colouring agent embedded in PP matrix and effect of different cooling conditions. At stage 1, CuO/PP and TiO_2/PP shows good distributions particles and good antimicrobial testing which were then used in next stage. PP/CuO/TiO_2/C had been selected as the best sample because of well distribution AA in PP matrix, low crystallinity and good antimicrobial resistance. Quenching method in warm water made PP/CuO/TiO_2/C sample more amorphous and increase antimicrobial efficacy. Conclusively, PP/CuO/TiO_2/C has high potential to reduce infection caused by microbes. It also can be used as new replacement to current catheter in biomedical application.

CHAPTER 1

INTRODUCTION

1.1 Introduction

A microbe or microorganism is an individual living animal or plant that is too small to be seen by naked eyes and can only be seen with aid of a microscope. Examples of microbes include algae, fungi, protozoa, bacteria and viruses. Microbes are found literally everywhere in the environment. Some species of them are pathogenic which can cause cross infection, allergic reactions, respiratory disorders, fouling, stains and degradation (Rosdahl and Kowalski, 2008).

Pathogen can cause two categories of diseases which are microbial intoxications and infectious diseases. A microbial intoxication is a disease that follows ingestion of a toxic that was produced in vitro by a pathogen. Food borne botulism is an example of microbial intoxication. On the other side, infectious diseases are diseases that follow colonization of the body by a pathogen. By following colonization, the pathogen proceeds to cause in vivo disease. According to this study, catheter is more exposed to infectious diseases which can cause in vivo disease (Engelkirk and Duben-Engelkirk, 2008, Charney, 2012).

Microbes infection has become a major health care problem in medical field causing an adverse impact on the quality of life of patients, and there are many alternatives and solutions that have been proposed to overcome the problems regarding this situation (Burke, 2003). Infection happens when a person is being harmed by microbe. Lately in biomedical field, catheter which is prone to microbial infection becomes a major concern. Catheters play a crucial role in hospital care, particularly in

the transport of intravenous fluids and medication. It is a tube that is inserted in a number of body cavities or vessels for providing drainage or allowing injection of fluids. Catheters can also be used to allow access for surgical tools. Any patients with an indwelling catheter, either urethral or suprapubic will develop a catheter-related problem. Problems can range from relatively minor to major complications with serious consequences for the patient's health. It is acknowledged that infection of medical devices like catheter may cause allergic reactions, swollen, nausea, fatigue, fever, severe pain and dizziness (Trindade and da Silva, 2011). Figure 1.1 shows swollen and allergic reactions caused by catheter to human's skin.

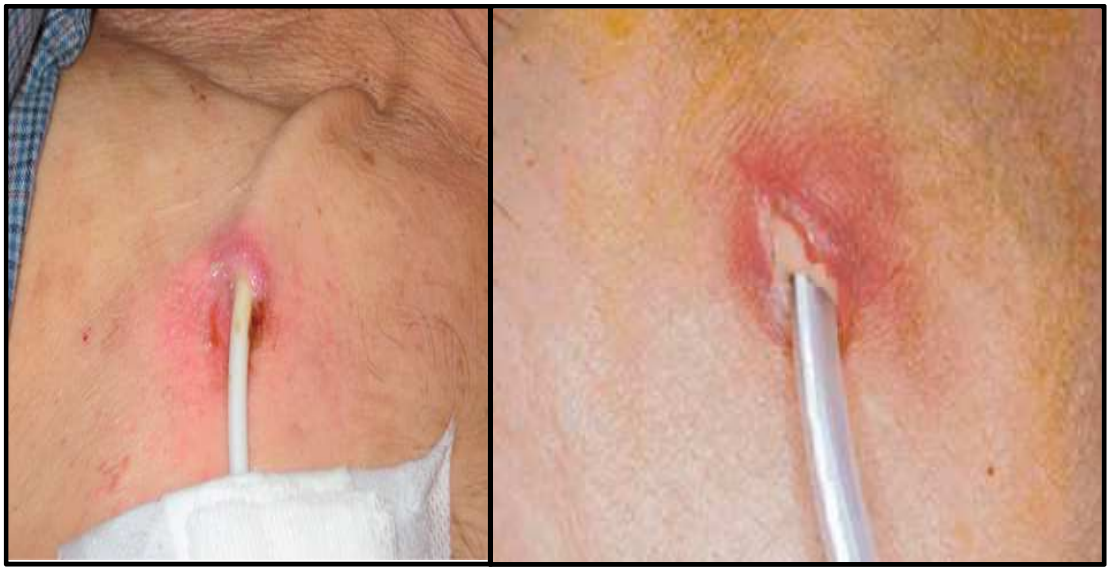

Figure 1.1: Swollen and allergic reaction caused by catheter to human's skin.

To minimize bio-contamination and reduce health hazards, elimination of the conditions in which these microbes thrive and, providing *in-situ* sterilization, holds immense significance. With the increasing concerns of healthcare, research and development in the field of biomedical and surgical appliances are being pursued extensively. In order to decrease the risk of microbial infection in catheter, antimicrobial agent is introduced. Antimicrobial agent plays an important role in the biomedical application in order to minimize the presence of microbes by killing microorganisms or inhibits their growth. This agent must be able to bind and inactivate a specific target inside microbes cell (Mieny, 2003). In this study, copper oxide and titanium oxide are used as antimicrobial agent.

Antimicrobial agents are classified into two groups which are organic and inorganic compounds (Arefi et al., 2012, Shukla et al., Fang et al., 2006). However, lately the organic antibacterial agents were not of interest for their poor antibacterial properties (Shukla et al.). Therefore, nowadays, inorganic antibacterial agents such as metal and metal oxides are more preferred due to their ability to withstand intensive processing conditions, high durability, selectivity and high resistance of heat (Hussain et al., 2006, Rosenzweig, 2003). Polymer matrix composite materials produced using metal oxides particles and polymers are the most promising type of materials applied in biomedical field for antimicrobial purposes (Hanna et al., 2011).

There are numerous ways by which antimicrobial properties can be accomplished in a polymer matrix. Incorporation of volatile and non-volatile antimicrobial agents directly into polymers, coating or adsorbing antimicrobials onto polymer surfaces, immobilisation of antimicrobials to polymers by ion or covalent linkages and the use of polymers that are inherently antimicrobial (Brody et al., 2010, Ishitani, 1995, Ozdemir et al., 1999, Gray et al., 2003, Appendini and Hotchkiss, 1997,

Appendini and Hotchkiss, 2002, Goldberg et al., 1990). Recently, metal oxides polymer matrix composite become more popular because of their high flexibility in designing, and better mechanical, chemical, electrical and optical properties as well (Altan et al., 2011).

1.2 Problem Statements

Historically, catheter has been made from variety of plastics including polytetrafluoroethylene (Teflon), silicone rubber, polyurethane and polyvinylchloride (PVC). Catheter made of Teflon seems to be easily kink and its stiffness properties can lead to endothelial injury and vascular mural thrombi. For PVC catheter, the limitations include contribute to high thrombogenicity, has high friction and drugs can easily adsorb to the plastic. Silicone rubber which has smooth and soft characteristics means that surgical incision and dissection is needed for placement and predisposes them to fracture or rupture. Polyurethane has higher friction and may get brittle with increasing shelf age (King et al., 2008, Pettersson, 2001, Payne-James et al., 2001). The optimal material still remains unknown and still being studied by most researchers. Figure 1.2 shows example of catheter tubes used in hospital.

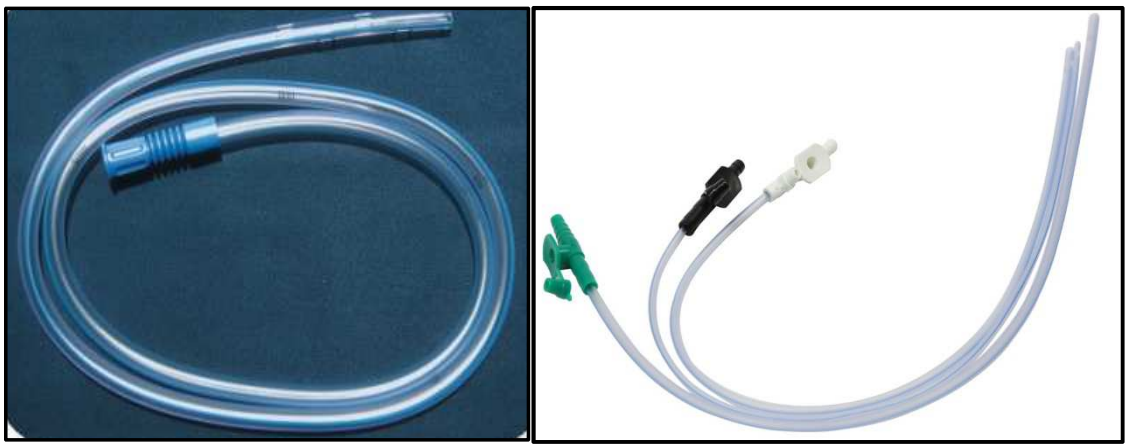

Figure 1.2: Catheter tubes used in hospital.

Currently, polypropylene also is the material of choice when comes to polymer based biomedical appliance material. This is because of the convenience offered by this polymer in terms of flexibility, film forming ability, light weight, chemical resistance and barrier properties. However, polypropylene is prone to bacterial infection causing an adverse impact on the health of patients. Under proper temperature and humidity conditions, plastics like polypropyle can be a good medium for the generation and the propagation of microorganisms which can cause irritations and infections (Palomba et al., 2012). Therefore, transformation of those polymers from plain biomedical appliance material to more specialized functional materials imparting antimicrobial and protective properties termed '*Active biomedical appliance material*', is of immense commercial interest to the medical industry.

Eventhough there are some potential AA that can be used to incorporate into PP matrix like CuO, TiO_2, Ag, $AgNO_3$ and ZnO, this research will be more focused on CuO and TiO_2 particles. However, testing regarding Ag, $AgNO_3$ and ZnO types of AA still being carried on in this study. Previous studies had proved the efficiency of CuO particles in resisting microbes attack. According to Delgado, Quidaja and Palma (2011), PP⁄CuOP composites present a higher release rate than PP⁄CuP composites in a short time, explaining the antimicrobial tendency. Copper metal (CuP) and copper oxide nanoparticles (CuOP) were embedded in a PP matrix. These composites present strong antimicrobial behaviour against E.coli bacteria that depends on the contact time between the sample and the bacteria. After just 4 hours of contact, theses samples are able to kill more than 95% of the bacteria. CuOP fillers are much more effective eliminating bacteria than CuP fillers, showing that the antimicrobial property further depends on the type of copper particle. Cu^{2+} released from the bulk of the composite is responsible for this behaviour.

Photocatalytic activity of TiO_2 refers to the ability of a material to form electron-hole pairs upon absorbing electromagnetic radiation. The reductive and oxidative abilities of the electron-hole pairs can lead to the production of strong oxidizing agents applicable for microbial decontamination. TiO_2 always maintains its photocatalytic abilities and for nanoscale TiO_2, it has a surface reactivity that fosters its interactions with biological molecules such as phosphorylated proteins and peptides (Liang et al., 2006). Based on previous study, functionalization of TiO_2 nanoparticles with polymers with good conducting properties can be used to direct the charged electrons and electropositive holes away from the surface of TiO_2. Moreover, the addition of polymers that allow for a large internal interface area between the polymer and the TiO_2 particle aids in charge segregation and also prevents charge recombination (Wang et al., 2009). Bactericidal activity of TiO_2 nanoparticles, founded on their photocatalytic reactivity. The illumination of the particles leads to the generation of ROS that oxidize membrane lipids and cause disruption to the outer and cytoplasmic membranes of the bacteria by lipid peroxidation which leads to the death of the bacterial cells (Maness et al., 1999, Kikuchi et al., 1997, Arora et al., 2007).

The concept of incorporating antimicrobial agents directly into the biomedical appliance is a very attractive approach. Therefore, CuO, TiO_2, Ag, $AgNO_3$ and ZnO antimicrobial agents will be incorporated into polypropylene to study the antimicrobial properties. The optimum sample will be selected based on distribution of particles in PP matrix, degree of crystallinity and antimicrobial efficacy.

1.3 Objectives

This research was done to achieve the following objectives:

1) To study physical and thermal properties of AA-PP samples. Physical properties of the samples can be obtained by using optical microscope to see their internal structures and SEM to investigate their external morphology (texture). For thermal properties, DSC was used to characterize melting temperature and degree of crystallinity for each sample.
2) To study the antimicrobial efficacy of AA-PP samples. The antimicrobial efficacy can be acquired by using embedded method and colony count method. Both methods will shows which sample has high resistance towards microbes.
3) To obtain optimum AA-PP sample for antimicrobial application. The best sample will be selected based on the result of embedded method from stage 1 (effect of different types of AA which embedded in PP matrix), stage 2 (effect mixture two to three different types of AA and colouring agent which embedded in PP matrix) and stage 3 (effect of different cooling conditions). Other than that, the sample needs to have good distribution of particles in PP matrix and low degree of crystallinity. Then, the chosen sample will proceed to run colony count method which is more quantitative analysis to prove its effectiveness in killing the microbes.

1.4 Scope of the Study

This study was focused in determining the appropriate antimicrobial agent which can be incorporate with polypropylene to reduce microbes attack. The relationship between distribution of AA particles in PP matrix, heat treatment effect and crystallinity of sample with antimicrobial properties will be discussed in this study. Characterizations used were optical microscopy observation, scanning electron microscope (SEM) microstructure observation, crystal structure analysis using X-ray diffraction (XRD), thermal properties by differential scanning calorimetry (DSC) analysis and antimicrobial testing. For antimicrobial testing, two methods had been used that includes disk diffusion testing and colony count testing. Both methods were used to study the effectiveness of AA-PP sample in resisting microbe attacks.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter provides the important information regarding the antimicrobial agent materials which include CuO, TiO_2 and PP together with the common antimicrobial testing like disk diffusion testing and colony count method. Basically, the basic fundamentals, feasibilities, and findings from previous studies of antimicrobial agents are discussed in this chapter. The information in this chapter is critically reviewed to short list the type of antimicrobial agent (AA) which is suitable to use for biomedical application.

2.2 Polypropylene

Polypropylene can be defined as any of various thermoplastic resins that are polymers of propylene (Maier and Calafut, 2008). It is an important thermoplastic polymer which compatible with many processing technique and used in wide variety of commercial applications, including automobile parts, medical devices, drug delivery systems, appliance parts, textiles, packaging and many more (Chen and Institute, 2007).

Polypropylene (PP) was first produced by Natta, following the work of Ziegler, by the polymerization of propylene monomer in 1954. Ziegler-Natta catalysts are the most common commercial catalysts in polypropylene polymerization. It was first polymerized from organo-metallic catalysts based on titanium and aluminium. The resulting semi-crystalline polymer has strong mechanical properties which explain its

rapid industrial development from its introduction into the market by Hoechst in 1965 (Chen and Institute, 2007, Tripathi, 2002, Karger-Kocsis, 1995).

Polypropylene (PP) has some advantages that make it special compared to other materials. Cost effectiveness and good performance of polypropylene determine its excellent growth rate. PP is considered as one of the lightest thermoplastics. It has the highest melting temperature of all commercial thermoplastics with better heat resistance compared to other low cost thermoplastics. PP has higher stiffness at lower density. It also has very good fatigue resistance as its oriented thin film can withstand more than one million repeated flexing in testing. In addition to this, PP offers good chemical resistance, can be recycled easily, good environmental stress cracking resistance, good hardness, ease of machining and also good processibility. Other than that, PP can be processed by many different methods, including injection moulding, blow moulding, extrusion, blown and cast film, and thermoforming. All these aspects make PP one of the most used polymers by industry (Tripathi, 2002, Chen and Institute, 2007). Table 2.1 shows properties of unmodified PP compared with other competitive thermoplastics.

Table 2.1: Properties of unmodified PP compared with other competitive thermoplastics (Tripathi, 2002).

Property	PP	LDPE	HDPE	HIPS	PVC	ABS
Flexural modulus (GPa)	1.5	0.3	1.3	2.1	3.0	2.7
Tensile strength (MPa)	33	10	32	42	51	47
Specific density	0.905	0.92	0.96	1.08	1.4	1.05
Specific modulus (GPa)	1.66	0.33	1.35	1.94	2.14	2.57
Maximum continuous use temperature (°C)	100	50	55	50	50	70
Cost (£/tone)	660	730	660	875	905	1550

2.2.1 Chemical structure

Polypropylene is synthesized by polymerizing propylene molecules, which are the monomer units in the presence of a catalyst under controlled heat and pressure. Propylene is an unsaturated hydrocarbon which is a gaseous byproduct of petroleum refining. Figure 2.1 below shows molecular structure of polypropylene.

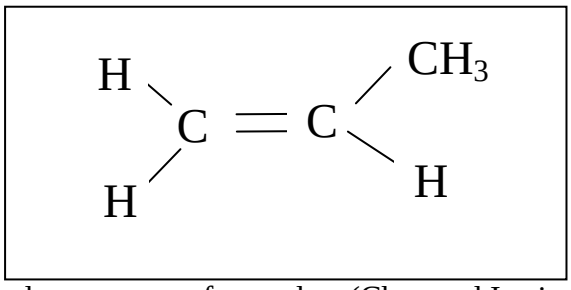

Figure 2.1: Molecular structure of propylene(Chen and Institute, 2007).

During reaction of polymerization, propylene molecules are joined one by one to form one polypropylene long chain. First, propylene reacted with transition metal catalyst to provide site for the reaction. Then, other propylene molecules are added sequentially through a reaction between metallic functional group on growing polymer chain and the unsaturated bond of the propylene monomer. One of the double bonded carbon atoms of incoming propylene molecules insert itself into the bond between the metal catalyst and the last carbon atom of polypropylene chain. Thousands of propylene molecules can be added sequentially to form a long polymer chain until the chain reaction is terminated (Chen and Institute, 2007). Figure 2.2 below shows polymerization reaction of propylene in the presence of an organometallic catalyst.

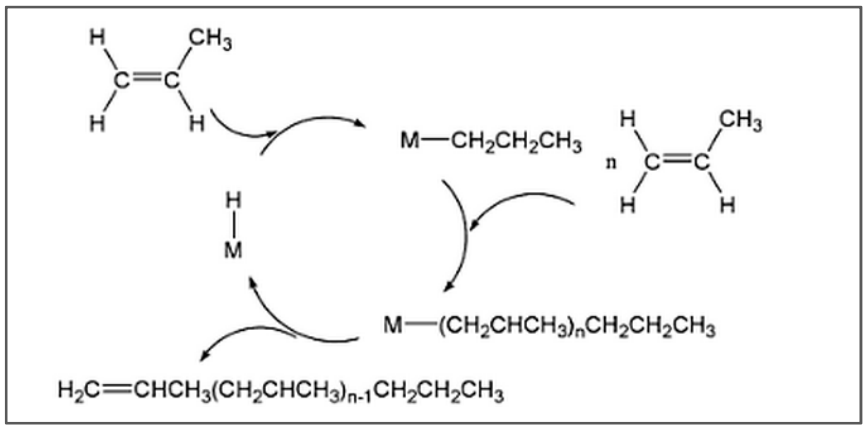

Figure 2.2: Polymerization reaction of propylene in the presence of an organometallic catalyst (Chen and Institute, 2007).

2.2.2 Antimicrobial properties of polypropylene (PP)

Polypropylene (PP) possesses superior mechanical resistance, abrasion resistance and has high resistance to chemical or biological agents. However, PP is still considered to be prone to microbes attack. Additives may impart antimicrobial activity of PP. Some recent post-spinning treatments to PP fibers such as plasma exposure, chemical modification and metal or metal oxide deposition have been applied to increase the reactivity, abrasion resistance, or wettability of the surface though possible drawbacks to these approaches. These actions contribute to changes in morphology, loss of polymer mechanical strength, and increase in web permeability (Arvidson et al., 2012). Thus, the actions will give poor effects to PP properties and contribute to less effective microbes attack.

2.3 Antimicrobial Agent

Polymers and metals have been extensively used in the field of biomaterials and can be tailored to suit numerous applications such as catheters, guidewires, oxygenators, blood filters stents and other implants. Typical examples of these materials are nylon, Pebax, polypropylene, polycarbonates, polyurethane, polyvinylchloride, polyesters and stainless steel. However, the biocompatibility and the physical properties of these materials are often less than ideal and can exhibit deficiencies such as poor haemo-compatibility, poor tissue compatibility, excessive complement activation, sensitivity to bacterial colonization and high friction force. As a result, development of novel surface modification and coating technologies are in progress to improve the biocompatibility and performance of a wide range of biomaterials used in medical devices (Trindade and da Silva, 2011).

Metals have been used for centuries as antimicrobial agents. The selection of an antimicrobial agent depends primarily on its activity against the target microorganisms (Yam, 2010). Silver, copper, gold, titanium and zinc have attracted particular attention by each having different properties and spectra of activity. With respect to nanoparticulate metals, the antimicrobial properties of silver and copper have received the most attention. Both of these materials have been coated onto or incorporated into various base materials like PMMA and hydrogels. There is an inverse relationship between size of nanoparticles and antimicrobial activity. Particles in the size range of 1-10nm have been shown to possess the greatest biocidal activity against microbes. Indeed, it has been shown that smaller silver nanoparticles are more toxic than larger particles. At nanoscale, Ag^+ ions are known to be released from the surface. The antimicrobial activity of small (<10nm) nanosilver particles is dominated by Ag^+ ions while for larger particles (>15nm) the contributions of Ag^+ ions and particles to the

antimicrobial activity are comparable, the Ag^+ ion release being proportional to the exposed nanosilver surface area (Subramani et al., 2012).

Research efforts are driven towards the adaption of conventional polymers to impart physic-chemical properties that can lead to multi-functional materials. The focus on polymer nanocomposites promises to provide cost effective alternative to suitably address the challenges involved. The integration of polymer and inorganic materials has constructed an attractive field in materials science. Dispersion of metal/metal oxide nanoparticles in polymeric materials has proved to be an efficient method to improve upon or add to the already existing properties of the bulk polymer material. The unique size or shape dependent on characteristics of the nanoparticles can alter the performance of polymer manifold. The changes are reflected in terms of enhanced mechanical strength, elasticity, optical properties, electrical conductivity and biological applicability including antimicrobial action (Trindade and da Silva, 2011). Table 2.2 below shows examples of nanocomposites exhibiting specific antimicrobial activity.

Table 2.2: Examples of nanocomposites exhibiting specific antimicrobial activity (Trindade and da Silva, 2011).

Nanocomposite material	Antimicrobial activity targets
Triglyceride oil based polymer-Ag nanocomposites	Gram-positive (S.aureus), Gram negative (P.aeruginosa) and spore forming (B.subtilis) bacteria.
Ag/polyrhodanine	Gram-negative (Escherichia coli), Gram-positive (Staphylococcus aureus and Candida Albicans)
Ag/TiO_2	Gram-negative (Escherichia coli), Staphylococci
Polystyrene/ TiO_2	Gram-negative (Escherichia coli), Gram-positive (S.aureus)

Among the antimicrobial metals, the use of copper (Cu) has a long tradition and even ancient civilizations gain benefits from its excellent antimicrobial effects. Nowadays, bacterial inactivation with Cu covers a wide spectrum including Cu-surfaces, Cu-nanoparticles, or release of Cu-ions. Bacteria interactions with materials are very complex and can be influenced by various factors such as surface charge, hydrophobic/hydrophilic interactions or surface heterogeneities (Klein et al., 2012, Kumar and Münstedt, 2005a, Matthews et al., 2010).

A number of features of the antimicrobial agent and polymer matrix can influence the degree of solubility, including: (i) polarity; (ii) hydrophobicity/hydrophilicity; (iii) crystallinity; (iv) differential solubility of the polymer and antibacterial agents indifferent solvents; and (v) differential melting temperatures, whereby the requirement for excessive heat to disperse one agent can result in the degradation of the other. Consequently, variation in the release profile of the antimicrobial agent between samples can occur if there is inadequate dispersal of the chemical agent within the polymer matrix (Hart et al., 2010).

A recent study shows that impregnation method, instead of coating the medical device like catheter with nanoparticulate silver, improved the antimicrobial activity of the device. This is due to the slow and continual release of silver ions that prolonged the antimicrobial effect (Li et al., 2006). However, another important advantage of impregnation is the protection of both inner and outer surfaces of catheters against bacterial colonization. Material is overall protected and thus can last longer from microbes attack (Li et al., 2006, Furno et al., 2004, Wilcox et al., 1998, Darouiche et al., 1999). Darouiche et al. (1999) also reported that use of central venous catheters impregnated with either minocycline and rifampin or chlorhexidine and silver sulfadiazine reduces the rates of catheter colonization and catheter-related bloodstream

infection as compared with the use of unimpregnated catheters. In this study, impregnated method was chosen to incorporate AA metal into PP rather than coating the polymer surfaces as it offers more advantages.

2.4 Types of Antimicrobial Agent

The metal oxides filled materials have to release the metal ions to a pathogenic environment in order to be efficacious. The entire process of metal ion release from metal oxides filled material is composed of three elementary processes. The processes include diffusion of water into the composite specimen, the reaction between the metal oxides and water molecules leading to the formation of metal ions and the migration of metal ions through the composite specimen leading to the release from the composite specimen to the aqueous environment (Kawashita et al., 2000, Kumar and Münstedt, 2005b).

2.4.1 Copper Oxide

Copper oxide is presumed to exert its antimicrobial effect towards microorganisms through inactivating functional groups in proteins by combining the –SH groups of enzymes and its microbe's inhibitory mechanism exactly same as an antimicrobial mechanism of nano-silver particles (Yoon et al., 2007). The schematic overview of the different pathways inducing cellular toxicity by CuO nanoparticles is shown in Figure 2.3.

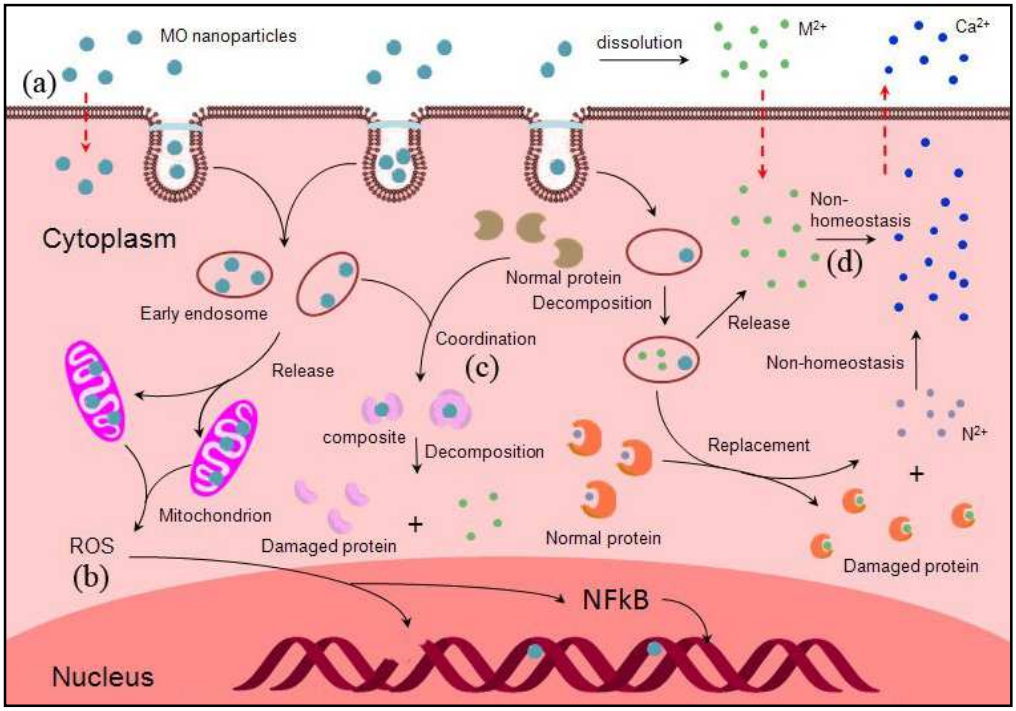

Figure 2.3: Schematic overview of the different pathways inducing cellular toxicity by CuO nanoparticles (Chang et al., 2012). (a) Potential mechanisms of CuO nanoparticles entry into cells; (b) The reactive oxygen species (ROS) effect of intracellular CuO nanoparticles (c) The coordination effect of Cu^{2+} and released from nanoparticles in cell; (d) The non-homeostasis effect disrupted by Cu^{2+}.

Copper oxide has higher temperature superconductivity, electron correlation effect, spin dynamics, higher durability, higher chemical and physical stability, very good in absorption and penetration (Ren et al., 2009, Issa et al., 2012). Furthermore, it is considered cheaper than silver, easily mixed with polymers, can be recyclable without loss of properties, ease of use widely available and long lasting product (Ren et al., 2009). It can be prepared with unusual crystal structure and high specific surface area for certain antimicrobial application (Ravishankar Rai and Bai, 2011). Polypropylene (PP) matrix embedded with CuO particles (40 nm) showed better dispersion of CuO

nanoparticles in PP matrix and strong antimicrobial action against E.coli within 4 hour with 99.9 % of bacterial reduction (Delgado et al., 2011).

CuO nanoparticles are more effective in killing gram-negative bacteria such as E.coli when compared to the Cu nanoparticles due to their highest oxidation state in naturally (Delgado et al., 2011). However, CuO nanoparticles were found to be most toxic against few tested microbes which are *Escherichia coli, Bacillus subtilis,* and *Streptococcus aureus* when compared to ZnO (except S.aureus), NiO, and Sb_2O_3 nanoparticles (Baek and An, 2011, Karlsson et al., 2008). CuO nanoparticles with sizes ranging from 20 to 95 nm also showed activity against several bacterial pathogens, including Staphylococcus aureus and E. coli (Delgado et al., 2011). Besides that, some researchers showed that, higher release rate of Cu^{2+} can be achieved by using CuO as antimicrobial filler when compared to Cu (Delgado et al., 2011). CuO fillers are much more effective eliminating bacteria than Cu fillers (Delgado et al., 2011).

Highly ionic copper oxide nanoparticles with higher concentrations were required to achieve maximum killing capability on wide range of bacterial pathogens when compared to the nano-Ag and nano-Cu (Ren et al., 2009, Ravishankar Rai and Bai, 2011). Polymer embedded with nano-CuO had lower contact-killing ability towards *Meticillin-resistant Staphylococcus aureus* (*MRSA*) strains (Ravishankar Rai and Bai, 2011). CuO nanoparticles were shown to cause of oxidative stress, cytotoxic, genotoxic, mitochondrial damage, and DNA damage, whereas this could not be observed for CuO micron-sized particles (Karlsson et al., 2008, Karlsson et al., 2009). High surface reactivity and high toxicity of CuO nanoparticles and also the release of Cu-ions lead to cell death and DNA damage (Karlsson et al., 2008, Karlsson et al., 2009).

Therefore, CuO nanoparticles were much more toxic than the Cu ions and the bulk form of CuO (Karlsson et al., 2008, Karlsson et al., 2009). Besides that, CuO nanoparticles were most potent regarding cytotoxicity and DNA damage when compared to the TiO_2, nanotubes, and ZnO (Karlsson et al., 2008). The Cu^{2+} ions which released from the polymer matrix composite is not responsible for any of the toxicity effect to human health but few studies showed that only copper nanoparticles and ionic copper are highly toxic to human and mammalian cells (Delgado et al., 2011).

Nano-copper (23.5nm) and ionic-copper (0.072nm) are moderately toxic and induce gravely toxicological effect and heavy injuries on kidneys, liver, spleen and stomach of experimental mice (Liu et al., 2009, Meng et al., 2007, Chen et al., 2006). But, micro-copper (17µm) is practically non-toxic and not induce any toxicological effect and heavy injuries on kidneys, liver, spleen and stomach of experimental mice (Liu et al., 2009, Meng et al., 2007, Chen et al., 2006). It has much smaller specific surface area as 3.99 $cm^2/g \times 10^2$ cm^2/g, which is about 1/940 to the nano-copper. It is also chemically inert due to lower specific surface area (Meng et al., 2007). The toxicological effect and injuries on kidneys, liver, spleen and stomach of experimental mice for different size of copper is shown in Figure 2.4 to 2.6. Therefore in this work, micron size CuO is introduced in PP to determine the antimicrobial properties.

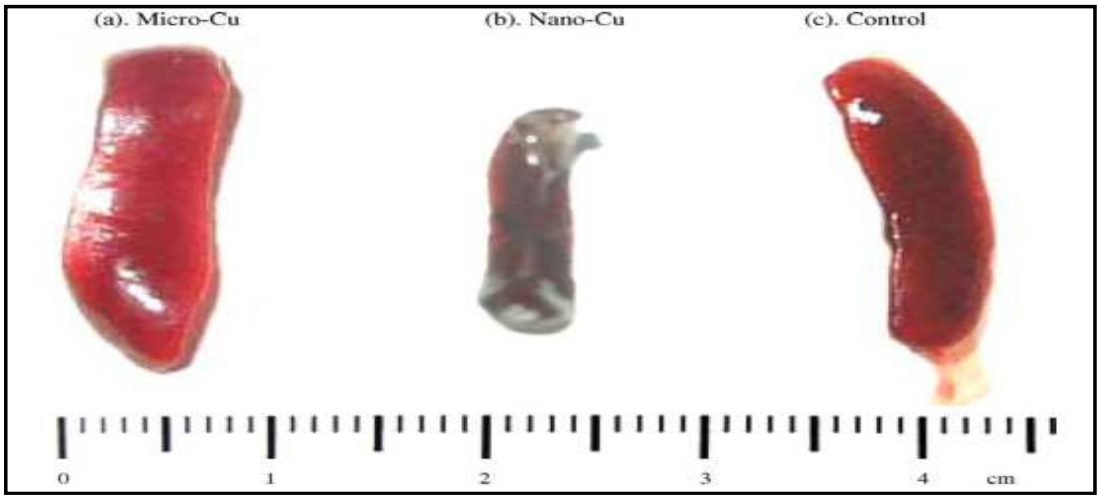

Figure 2.4: The appearance of spleens of experimental mice (Chen et al., 2006).

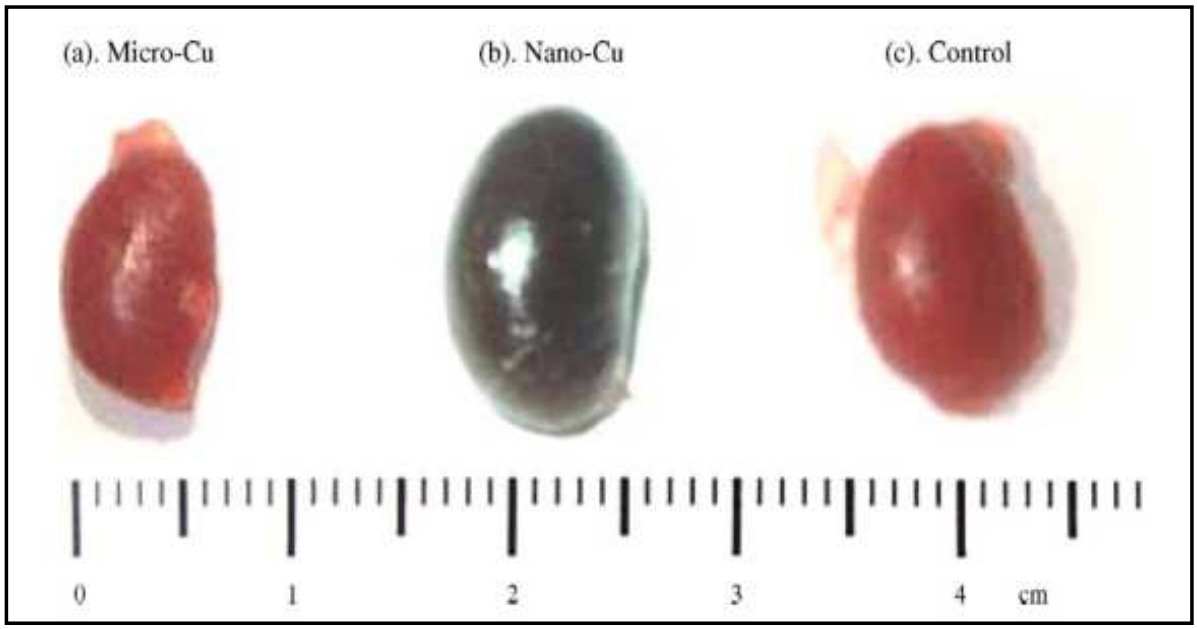

Figure 2.5: The appearance of kidneys of experimental mice (Chen et al., 2006).

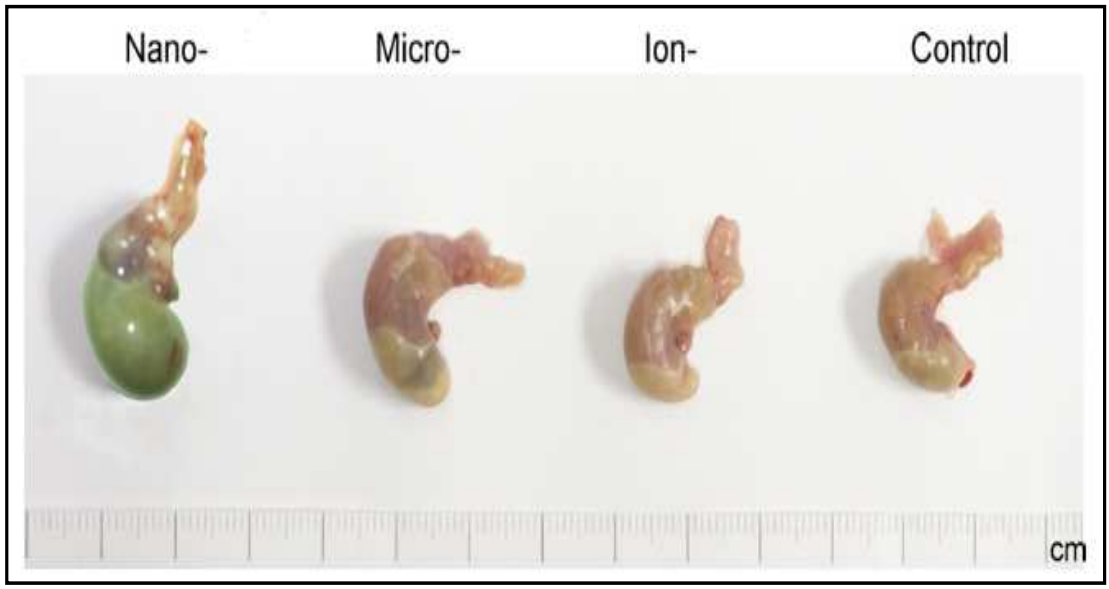

Figure 2.6: The appearance of mice's stomach after single oral gavage for 24 hours (Meng et al., 2007).

2.4.2 Silver

The inhibitory action of nano-silver against microbes could be seen through the dual mechanisms of denaturation and oxidisation where silver ions attack some functional groups in proteins by combining the –SH groups of enzymes and generate reactive oxygen causes cell death of bacteria (Wasif and Laga, 2009, Ruparelia et al., 2008, Yoon et al., 2007, Radheshkumar and Münstedt, 2005). Silver is a long lasting biocide with high temperature stability and low volatility (Cavalu et al., 2011). Silver is a superior antibacterial agent in eliminating bacterial colonies of *S. aureus* and *E. coli* when compared to copper and titanium (Kaali, 2011, Ruparelia et al., 2008). The silver ion release rate depends on level of water uptake to release Ag^+ ions. The high crystalline Ag-PA composite has lower level of water uptake and Ag^+ ions release (Damm et al., 2008). Therefore, amorphous polymer is preferable to enhance the Ag^+

release (Damm et al., 2008, Radheshkumar and Münstedt, 2005). Besides, crystallinity, Ag^+ ions release was found to be dependent on specific surface area.

For instant, silver nanoparticles with lower specific surface area of 0.78 m^2/g in polyamide matrix had finer dispersion and higher release of silver ions compared to PA/Ag composites which having SSA of 1.16 and 2.5 m^2/g in PA matrix had agglomerated region in matrix which act as barrier for more silver ions release (Radheshkumar and Münstedt, 2005, Kumar and Münstedt, 2005b). Silver showed highest inhibitory activity against gram negative bacteria such as *E. coli* than gram positive bacteria such as S. aureus (Ravishankar Rai and Bai, 2011, Cavalu et al., 2011, Matthews et al., 2010, Sourav Ghosh, 2010). But, B.substilis was more sensitive than *E. coli* compared to silver nanoparticles (Kim et al., 2007, Yoon et al., 2007). Silver (25 nm) nanoparticles smaller size and high surface area has highest antibacterial effect compared silver microparticles (Hamouda, 2012). Silver has strong effect on both gram positive & gram negative bacteria and several species of bacteria (Ashe, 2011). Silver kills bacteria by strangling them in a warm and moist environment. Silver is 3–4 times more active at pH8 than at pH6 (Wasif and Laga, 2009). The use of silver nanoparticles may cause of toxicity for humans and mammalian cells (Hamouda, 2012, Sambhy and Sen, 2008, Karlsson et al., 2008, Trindade and da Silva, 2011, Linkov and Steevens, 2009, Raffi et al., 2008, Radheshkumar and Münstedt, 2006). Lately, the toxicity of using micron sized silver particles in medical field is less reported. This is probably due to the fact that the antibacterial efficacy of AA-PP composite is reduced using micron sized of antibacterial agent in the polymer matrix (Hamouda, 2012).

2.4.3 Titanium oxide (TiO_2)

The photoexcited TiO_2 particles under UV illumination produce activated electrons and holes which strongly react with atmospheric water and oxygen in order to create ROS, such as active free hydroxyl radicals (-OH) that oxidize membrane lipids and cause disruption to the outer and cytoplasmic membranes of the bacteria by lipid peroxidation; this leads in turn to the death of the bacterial cells (Ravishankar Rai and Bai, 2011, Arora et al., 2007, Adil M Allahverdiyev, 2011). The photocatalysis mechanism of titanium dioxide and bacteria inactivation in the presence of an aqueous suspension of different sized TiO_2 particles are shown in Figure 2.7 to 2.8.

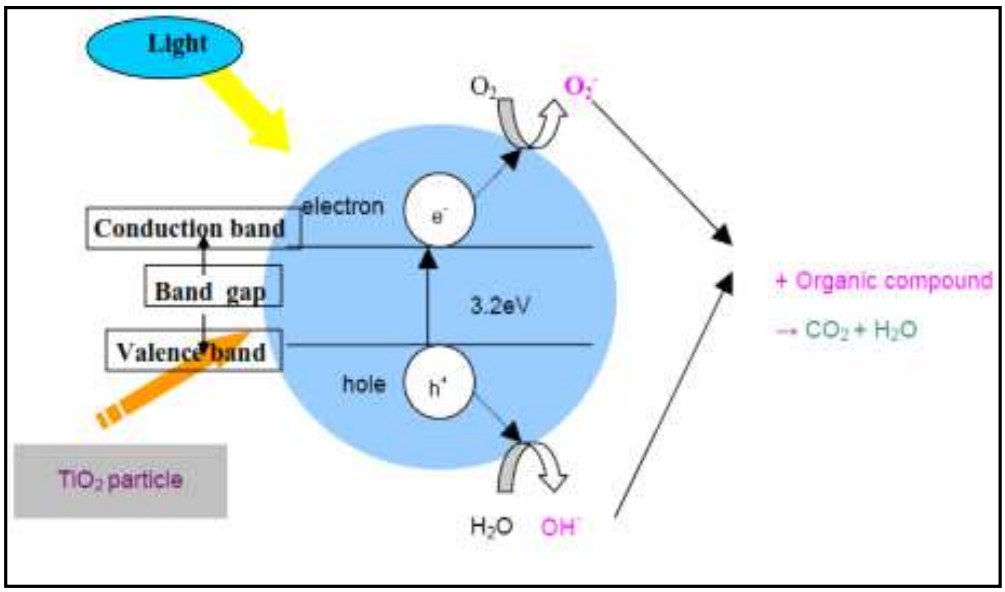

Figure 2.7: Photocatalysis mechanism of titanium dioxide (Al-Anbagi et al., 2011).

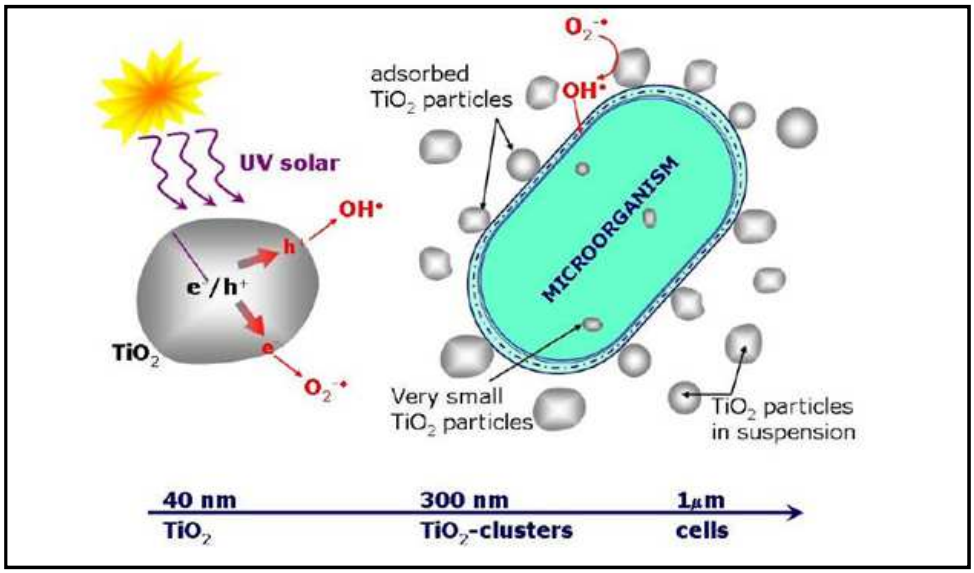

Figure 2.8: Bacteria inactivation in the presence of an aqueous suspension of different sized TiO_2 particles (Al-Anbagi et al., 2011).

Titanium oxide (TiO_2) is a noncombustible and odorless white powder, naturally exists in anatase, rutile and brookite (Wang et al., 2007). Titanium oxide exhibits high modulus of elasticity, high resistance to corrosion, high durability, poorly soluble matter, excellent biocompatibility, light weight, strong oxidizing agent, excellent balance of mechanical properties, high melting point (1668°C), high specific strength, hard, biologically inert, low wear and abrasion resistance, safe materials to human beings and animals, broad spectrum antibiosis, antibacterial, self - cleaning, UV protection, hydrophilic or ultra–hydrophobic, high chemical stability, low cost, non-toxic at microscale and gains clean photocatalytic properties when irradiated with UV light (Davis, 2003, Acosta-Torres et al., 2011, Trindade and da Silva, 2011, Adil M Allahverdiyev, 2011, Wang et al., 2007, Dastjerdi and Montazer, 2010, Kale, 2011).

TiO_2 showed maximum antibacterial activity towards gram-negative bacteria E.coli and minimum antibacterial activity against the fungi Candida albicans (Mukherjee et al., 2011, Arora et al., 2007). Titania only exhibits strong antibacterial effect towards bacteria, viruses and fungi in the presence of near UV light (Mukherjee