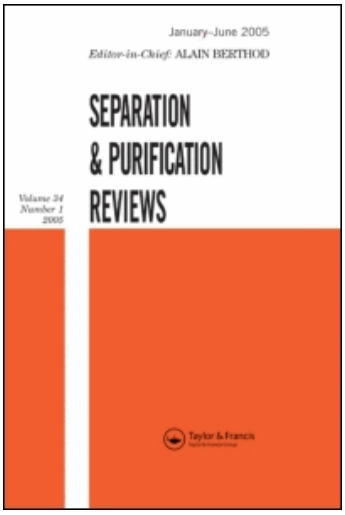

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APPLICATION OF THE PHOTOCATALYTIC CHEMISTRY OF TITANIUM DIOXIDE TO DISINFECTION AND THE KILLING OF CANCER CELLS

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Abstract

This article will review the work that has been published on disinfection and the killing of cancer cells using photocatalytic chemistry with titanium dioxide (TiO_2). This is an application of photocatalytic chemistry that has been under active investigation since 1985. Because the nature of the research is such that it brings together disparate disciplines, this review provides background on photocatalytic chemistry, fundamental characteristics of target organisms, potential applications, and the toxicology of titanium dioxide. Literature identified in searches done through September 1998 is included.

Introduction

This article will review the work that has been published worldwide on disinfection and the killing of cancer cells using photocatalytic chemistry with titanium dioxide (TiO_2). Health effects of titanium dioxide are also covered since applications of photocatalytic technology will bring a range of organisms into contact with crystalline and particulate forms of the photocatalyst. This is an application of photocatalysis that has been receiving increasing attention since the first report of microbiocidal effects by Matsunaga, Tomada, Nakajima, and Wake in 1985.[1] The research community that has been most active in the study of photocatalytic chemistry is composed mainly of chemists and chemical engineers who are often unfamiliar with the terms used by the microbiologist. Microbiologists for their part may be less familiar with the photochemistry and reactor issues associated with photocatalytic systems. For this reason this review will provide a brief introduction to the structure of target organisms and to current practices for disinfection as required to discuss the interaction of microorganisms with activated photocatalyst surfaces and the chemical species produced when titanium dioxide

surfaces are irradiated. Patent literature that includes claims related to this topic is also included. Titanium dioxide has been used extensively as a white pigment and as a cosmetic ingredient. Literature that studies the potential health effects of TiO_2 exposure is also included in this review.

Photocatalytic chemistry of titanium dioxide has been extensively studied over the last 25 years for removal of organic and inorganic compounds from contaminated water and air and for the partial oxidation of organic compounds. The most active photocatalysts are formulations based on the anatase crystal phase, and most work has been done using the P25 form of TiO_2 produced by Degussa Chemical Company (Germany). This material is a mixture of phases with an approximate composition of 75% anatase and 25% rutile and has a BET surface area of about 50 m^2/g. The literature for the photocatalytic oxidation or reduction of organic and inorganic compounds has been the subject of comprehensive bibliographies[2,3,4] and numerous reviews.[5,6,7,8,9] Engineering requirements for practical photocatalytic systems have been discussed.[10] A recent review includes some coverage of the application of photocatalytic chemistry to disinfection.[11] Work by Japanese groups has been included in short reviews.[12,13,14]

Background

There are many circumstances where it is necessary or desirable to remove or kill microorganisms found in water, air, on surfaces, or in a biological host. Disinfection of water is required for direct human consumption as well as in the production of products to be consumed by humans or animals. Disinfection of air is required in medical facilities, in production processes where biological contamination must be prevented, and in facilities that raise or care for experimental animals and plants that may be sensitive to infectious agents. Disinfection can require the removal or deactivation of pathogenic bacteria, viruses, protozoa, or fungi. The status of methods to disinfect drinking water has been reviewed.[15,16,17] This is a particular challenge in rural areas of developing countries. The most widely used methods in developed countries are chlorination and ozonation, but germicidal lamps (low pressure mercury vapor lamps emitting at 254 nm) are being evaluated and used on a large scale. Disinfection of air can be accomplished by the use of germicidal lamps[18] or size exclusion filters (high efficiency particle air, HEPA, filters).[19,20,21] Biocides are widely used for control of biofilm growth in cooling towers and chilled water systems. Surfaces can be disinfected by ozone exposure, irradiation with UV light,

TABLE I. Modes of microbe removal or killing action for various disinfection methods

Method	OH	O_2^-, H_2O_2	Cl	hυ	Adsorption	Trapping
UV (254 nm)				x		
Chlorine			x			
TiO_2 (300-380 nm)	x	x		(a)	x	(b)
TiO_2 (254 nm)	x	x		x	x	(b)
HEPA filter						x

(a) Near ultraviolet light may have some killing effect on sensitive organisms.
(b) In some catalyst configurations the titanium dioxide layer may act as a particle filter.

washing with disinfectants, or application of heat.[22] Some of the methods relevant to this review are summarized in Table I and compared with the modes of action proposed for TiO_2 systems.

Photocatalytic methods are unique in having several modes of action that can be brought to bear on disinfection. The target of disinfection processes are pathogenic organisms including viruses, bacteria, fungi, protozoa, and algae. Each presents a challenge in terms of the structure and defense mechanisms that must be overcome. The current disinfection technologies rely on chemical or photochemical induced damage or physical removal by filtration. Mechanisms for the killing of cells by conventional methods have been covered in earlier reviews.[15,16,23,24,25] Irradiation from germicidal lamps, 254 nm, results in cross-linking of thymine groups in DNA. Free radicals such as chlorine atom or hydroxyl radical, $OH^{\bullet}$, can result in DNA strand breakage or initiate autoxidation of lipids or other cell components. Ozone or singlet oxygen can attack molecular structures found in cell components with some selectivity.

Organisms have evolved defense and repair mechanisms to overcome photochemical and oxidative damage that allow them to live in an aerobic environment and to deal with the low levels of UV radiation found in sunlight. All life forms are sensitive to damage to DNA macromolecules, therefore, nature has equipped cells with several defense and repair mechanisms against such damage. In particular ultraviolet light in the germicidal range around 254 nm causes the adjacent thymine bases on a DNA strand to form a thymine dimer, thus blocking protein synthesis and disabling the proper replication of the DNA helix during cell division cycle. Cells then respond by either undergoing a photochemical repair using a photolyase enzyme, or by dark excision repair using endonuclease, exonuclease, DNA polymerase, and ligase enzymes.[24,26,27]

It is well documented that an increased pressure of oxygen is toxic to all forms of life, and the production of oxygen radicals is the root cause of oxygen toxicity. During respiration the stepwise reduction of oxygen leading to H_2O generates the reactive intermediates superoxide radical ($O_2^{\cdot}$) and hydrogen peroxide (H_2O_2), both of which are reactive toward biological macromolecules and can be precursors for hydroxyl radical.[28] Therefore, all organisms prevailing in the aerobic atmosphere have evolved a protective mechanism to scavenge both $O_2^{\cdot}$ and H_2O_2 to a very low steady-state concentration.

Indeed, all aerobic life forms are reported to have a superoxide dismutase (SOD) enzyme to dismutate $O_2^{\cdot}$ to H_2O_2 and O_2, and a catalase enzyme to convert H_2O_2 to H_2O and additional O_2 as innocuous end products.[29,30] Several procaryotes including *E. coli*, have evolved to produce different forms of SOD strategically distributed within a cell to shield the cells from any oxidative damage resulting from their normal metabolism. In *E. coli*, an Fe-SOD is distributed along the periphery of the cytoplasm close to the inner membrane to dissipate any radicals produced from aerobic respiratory pathways. A second cytoplasmic Mn-SOD is more abundant in the central region of the cell where the nucleoid is in order to protect DNA from damage caused by oxidants. A third Cu,Zn-SOD, found exclusively in the periplasm, is proposed to guard the cells from any exogenous source of $O_2^{\cdot}$, whether produced from the immediate hostile environment or by other phagocytic cells.[25]

In some cases it is not sufficient to kill or remove the microorganism responsible for a pathogenic or allergic response. Some bacteria produce endotoxins and exotoxins. Some Gram-positive bacteria and, less commonly, Gram-negative bacteria release exotoxins into the medium of the growing culture. These are most often heat sensitive proteins. Endotoxins are most frequently produced within Gram-negative bacteria and are not released unless the outer membrane becomes damaged. Endotoxins are usually lipopolysaccharides. Contact with these compounds can give rise to medical problems, and allergic responses can be caused by cell structures that persist even after the cell is no longer viable.[31]

Mode of action of TiO_2

Two crystalline forms of TiO_2 have photocatalytic activity, anatase and rutile. Anatase has a band gap of 3.2 eV and for rutile it is 3.0 eV. Anatase has been found to be the most active form.

The action spectrum for anatase shows a sharp decrease in activity above about 385 nm. The photocatalytic process includes chemical steps that produce reactive species that in principal can cause fatal damage to microorganisms.[5,6,7,8,11] The steps are summarized in Table II and include formation of the following species: hydroxyl radical, hydrogen peroxide, superoxide, conduction band electron, and valence band hole.[32] Formation of singlet oxygen on irradiated TiO_2 has also been reported[33] but is not usually considered to be present under the usual conditions of disinfection reactions. The reactive oxygen species (ROS) may disrupt or damage various cell or viral functions or structures. The preponderance of evidence on photocatalytic chemistry in aqueous solution suggests that the hydroxyl radical formed by hole transfer does not diffuse from the surface of the TiO_2 into bulk aqueous phase.[34]

For a cell or virus in contact with the titanium dioxide surface there may also be direct electron or hole transfer to the organism or one of its components. If titanium dioxide particles are of small size, they may penetrate into the cell and these processes could occur in the interior. Since light is an essential component of the photocatalytic system, there can also be direct photochemistry as there would be from any UV source. There is also the possibility for enhanced or unique photochemistry resulting from the irradiation of the microbe while it is adsorbed on an oxide surface, as has been observed for molecules.[35] The relative sizes of molecular or biological targets of photocatalytic chemistry and the most commonly used form of TiO_2, Degussa P25, may have some significance. These are given in Table III. Orientation and distance effects are likely to be more pronounced in the case of microbes which are comparable in size to aggregates of the titanium dioxide particles.

Hydroxyl radicals are highly reactive and therefore short-lived. Superoxide ions are more long-lived; however, due to the negative charge they cannot penetrate the cell membrane. Upon their production on the TiO_2 surface, both hydroxyl radicals and superoxide would have to interact immediately with the outer surface of an organism unless the TiO_2 particle has penetrated into the cell. Compared to hydroxyl radicals and superoxide ions, hydrogen peroxide is less detrimental. However, hydrogen peroxide can enter the cell and be activated by ferrous ion via the Fenton reaction.

$$Fe^{+2} + H_2O_2 \rightarrow OH^{\bullet} + OH^{-1} + Fe^{+3}$$

TABLE II. Mechanism of a photocatalytic process on irradiated titanium dioxide

Electron-Hole Pair Formation

$TiO_2 + h\nu \rightarrow TiO_2^- + OH^\bullet$ (or TiO_2^+) (conduction band electron and valence band hole)

Electron removal from the conduction band

$TiO_2^- + O_2 + H^+ \rightarrow TiO_2 + HO_2^-$

$TiO_2^- + H_2O_2 + H^+ \rightarrow TiO_2 + H_2O + OH^\bullet$

$TiO_2^- + H^+ \rightarrow TiO_2 + \frac{1}{2} H_2$

Oxidation of organic compounds

$OH^\bullet + O_2 + C_nO_mH_{(2n-2m+2)} \rightarrow\rightarrow\rightarrow nCO_2 + (n-m+1)H_2O$

Nonproductive Radical Reactions

$TiO_2^- + OH^\bullet + H^+ \rightarrow TiO_2 + H_2O$ (recombination)

$2OH^\bullet \rightarrow H_2O_2$

$2HO_2 \rightarrow H_2O_2 + O_2$

$OH^\bullet + H_2O_2 \rightarrow H_2O + O_2$

$OH^\bullet + HCO_3^- \rightarrow CO_3^{\bullet-} + H_2O$

TABLE III. Relative sizes of TiO_2 particles and target or other representative species that may be present in media being treated

Species	Size, microns
Benzene Molecule	0.00043
TiO_2 Crystalite (Degussa P25)	0.03
TiO_2 Agglomerate (in water)	1-3
Virus	0.01-0.3
E. coli (rod shape)	1 X 3
Yeast Cell	1-5 X 5-30
Protozoa	1-2000
Atmospheric Dust	0.001-13
Tobacco Smoke	0.01-1

The ability of bacteria, such as *E. coli*, to sequester iron is well documented.[36,37] Iron levels on the cell surface, in the periplasmic space or inside the cell, either as iron clusters or in iron storage proteins (such as ferritin) are significant and can serve as a source of ferrous ion. Therefore, while the TiO_2 is being illuminated to produce H_2O_2, the Fenton reaction may take

place *in vivo* and produce the more damaging hydroxyl radicals.[38,39] When the light is turned off, any residual hydrogen peroxide would continue to interact with the iron species and generate additional hydroxyl radicals through the Fenton reaction. When both H_2O_2 and superoxide ion are present, the iron-catalyzed Haber-Weiss reaction can provide a second pathway to form additional hydroxyl radicals.[40]

$$Fe^{+3} + O_2^{\cdot} \rightarrow Fe^{+2} + O_2$$
$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^{-} + OH^{\bullet}$$

Since the initial actions of these reactive oxygen species (ROS) target the outer surface of a cell, the rigidity and chemical arrangements of their surface structure will determine how effectively the TiO_2 photocatalytic disinfection process functions.

Photocatalytic Reactor Configurations

Photocatalytic reactors are designed to operate in either a liquid-solid system (*e.g.*, water disinfection) or a gas-solid system (*e.g.*, air disinfection). The two systems have implications with respect to reactor design. Another important distinction is whether the catalyst is fixed, i.e. immobile within the system, or moveable. Therefore, photocatalytic reactors fall into four broad categories:[5,10,11]

1) Liquid-solid, moveable bed reactors. These systems typically involve slurries of TiO_2 suspended in the liquid to be treated. The concentration of TiO_2 typically ranges between 0.05% and 1% by weight. Light penetration limitations prevent the use of higher concentrations. The catalyst flows into and out of the reactor with the liquid being treated. Typically either natural or artificial irradiation sources are external to the system, and the photons are transmitted through UV-transparent ports. A subsequent separation step is necessary to remove the TiO_2 from the treated water.

2) Liquid-solid, fixed bed reactors. Due to lower reaction rates, these systems are uncommon. The relatively low extent of contact between the catalyst and the molecules to be oxidized leads to mass transport limitations in fixed bed aqueous systems.

3) Gas-solid moveable bed reactors. Fluidized bed systems have been studied for destruction of chemicals in air. The catalyst particles are not entrained in the air stream. Rather, in a properly designed system, the catalyst is contained in the irradiated reactor vessel. An entrained bed system could also be envisioned since separating the catalyst particles from the air stream is easier than separation from a water slurry. It has been postulated that a moveable bed reactor may benefit from the "light-dark" phenomena explored by Sczechowski and coworkers for the liquid-solid system.[41]

4) Gas-solid fixed bed reactors. Reactors of this type have been widely studied and fabricated in a variety of geometries. Annular reactors typically feature an inner annulus which is the light source or may have a UV-transparent sheath around the light source. The inside surface of the outer annulus is often coated with TiO_2 via a variety of methods, leading to even illumination of the coated surfaces. The annular region can also be filled with TiO_2 crystals or substrates coated with TiO_2. Other geometries include powder layer reactors in which the powder is supported on a frit, and the fluid to be treated flows normally through the powder and the frit. External illumination is provided through a UV-transparent window. UV-transparent tubes, filled with catalyst particles or coated with catalyst have also been externally illuminated with both natural and artificial light.

Structure of Target Organisms

The common microbes carried by indoor air or in a water stream are very diverse. Therefore, an understanding of the microbial morphology will aid in the design of more efficient photocatalytic technologies for specific disinfection applications.

Bacteria

Bacteria are procaryotic microorganisms that do not contain the nucleus characteristic of cells of higher plants and animals (eucaryotic cells). The DNA molecules do not have a nuclear membrane to separate them from the cytoplasm. The cytoplasm of procaryotic cells is not differentiated into distinguishable units for specialized functions; for example, respiration takes place on the cell membrane. Although there are thousands of different species of bacteria, most of them fall into three general morphologies: spherical, rod or spiral.[42] They range in size from 0.5 to 5 microns in maximum dimensions. Based on the different ability of their cell wall

components to be stained by the Gram stain, bacteria are divided into two classes: Gram-positive and Gram-negative organisms. Gram-positive bacteria have the classical cell wall, of 20 - 80 nm in thickness which is composed mainly of a peptidoglycan polymer. In many Gram-positive bacteria the thick peptidoglycan layer accounts for nearly 80% of their cell wall components. The rest of the components are 10 to 20% of teichoic acids, and minor amounts of lipids, proteins and lipopolysaccharides. The cell wall of the Gram-negative cells is chemically more complex. Here the peptidoglycan layer is thinner (2-6 nm thickness) and accounts for only 10% of the cell wall. The outermost layer of Gram-negative bacteria, the outer membrane, is about 6 to 18 nm thick and accounts for the rest of the cell wall. The outer membrane consists of 50% lipopolysaccharides, 35% phospholipids, and 15% lipoproteins. Together, the peptidoglycan and outer membrane provide mechanical protection to maintain intact cell morphology and determine antigenicity and sensitivity to phage infection, similar to the functions of Gram-positive cells. In addition, the outer membrane of Gram-negative cells influences permeability of many moderate or large size molecules. The added impairment of material accessibility through the outer membrane explains why under certain circumstances the Gram-negative bacteria are more resistant to many chemical agents than the Gram-positive cells.[43,44]

Underneath the cell wall of all bacteria lies the cytoplasmic or plasma membrane, which is about 7.5 nm in thickness and is composed of the phospholipid bilayer. The cytoplasmic membrane is extremely important in maintaining viability of cells. It has the unique property of selective permeability--allowing the passage of certain metabolites in and out of cells while excluding other compounds. In addition to maintaining osmotic equilibrium, the cytoplasmic membrane also contains the necessary enzymes for the synthesis, assembly, and transport of cell wall components. Perhaps most important of all, the procaryotic cell membrane contains the machinery for the electron-transport and oxidative phosphorylation reactions. The electron carriers and enzymes responsible for the redox reactions must be properly linked on the membrane in order to couple the free energy change to ATP synthesis. Therefore, any disruption to the cell membrane integrity will cause the discharge of membrane potential and impose detrimental effects on cell survival.[45] The cytoplasmic membrane also serves as the binding site to which bacterial DNA is anchored and it is also involved in the replication and segregation of DNA molecules during cell division.[46]

The periplasmic space in Gram-negative cells, and less distinctly in Gram-positive cells, lies between the cytoplasmic membrane and cell wall.[47] Many enzymes, proteins, and electron

mediators are present in this dynamic, gel like environment. Any materials that have gained entry through the outer membrane and are still too large to permeate through the cell membrane are processed by hydrolytic enzymes. Components of the cell wall and outer membrane are also turned over here, and newly made materials are shuttled through this space before reaching their final destination. Many electron mediators are positioned in this space and participate in energy-yielding reactions. Since this compartment is immediately affected by a microbe's external environments, it may play a critical role in defending the cell against foreign agents.

The exterior surface of some bacteria may have other structures and materials such as slimes, sheaths, s-layers, and cilia.[43] The slime layer or capsule of certain bacteria is very viscous and acts as a protective coating to the cell wall. The capsules can serve as stored food for the organisms and increase the infective capability for pathogenic organisms. The viscous slime layer also enables bacteria to colonize as biofilms on solid surfaces such as air filters, or to attach to dust particles and travel in air. Some or all of these structures may be formed in response to environmental stimuli and can be absent in bacteria cultivated in a relatively benign laboratory culture. These phenomena certainly will have a significant influence on their survival. Any treatment process would have to incorporate appropriate responses in the design to operate effectively.

Under severe environmental conditions certain bacteria, especially those from the genera of *Bacillus* and *Clostridium*, can produce spores within their cells (endospores). The bacterial spore contains a unique Ca^{+2}-dipicolinic acid-peptidoglycan complex, quite different from its parent vegetative cell.[48] It is believed that the nature of this complex, along with its dehydrated protoplast, and the extent of mineralization account for the spore's extreme resistance to many adverse physical and chemical agents.[49] Sporulation represents a dormant stage during the development of a cell's life cycle. Their durability allows them to survive for a long time while being carried by dust in air--only to germinate when nutrients become available.

The thick wall of spores is impermeable to most damaging agents. However, H_2O_2 and organic peroxides are reported to penetrate freely.[49] Most spores contain a high levels of transition metal, therefore, a Fenton-type reaction is likely to take place under this condition. The degradation of the outer coat by these radicals is not lethal to the subsequent germination of the de-coated spores. Nevertheless, once the protective coat is removed, the protoplast and its membrane become the target of direct radical attack, a lethal event. Therefore, destruction of spores by oxidative damage using the photocatalytic process is a viable concept.

Viruses

Viruses constitute a group of heterogeneous and much simpler organisms.[42] They range in size from 0.01 to 0.3μ, much smaller than bacteria. Viruses are unique in that they have no independent metabolic activities and have to rely solely on infecting living hosts to reproduce themselves. Unlike all other forms of life, viruses may contain either DNA or RNA as the genetic materials, but not both. The nucleic materials are surrounded by a protein coat to protect them from harmful agents in the environment. The protein coat also provides the specific binding site necessary for the attachment of a virus to its host. Some viruses also contain an outer envelope made up of lipids, polysaccharides, and protein molecules. The lipids and polysaccharides are of host cell origin, and their presence allows a virus to fuse with a host cell and thus gain entry.

A virus not having the outer envelope infects a cell in quite a different manner. Infection is initiated by the attachment of a specialized site on the surface of the protein coat of the virus onto a specific receptor site on the surface of the host cell. Once this binding is complete viruses can release genetic materials into the host cell and take advantage of the machinery of the host cell to reproduce and assemble themselves. These newly-produced viruses are now ready to infect other neighboring cells. Therefore, one of the key processes to disable viruses is through the control of their surface structures, especially their binding sites, so they can no longer recognize the receptor sites on the host cells. Since the TiO_2 photocatalytic process attacks most effectively on the cell surface, it may be a viable technology to disinfect viruses through modification of their surface structures.

Fungi

Fungi (including mold and yeast) are very diverse in their morphology.[42,50] Except for unicellular yeast, most fungi exist as multicellular filaments. Fungi are eucaryotic organisms (their genetic materials are enclosed in a nucleus surrounded by a membrane). Another feature of eucaryotic cell is that its cytoplasm contains many highly differentiated units—organelles, each of which performs a specific cellular function. For example, the respiratory pathway in eucaryotic cells is located in the organelle mitochondria. Fungi are heterotrophs, obtaining their food either by infecting a living host as parasites, or by causing the decay of dead organic matter. The most

common method of reproduction is by asexual spore formation. These air-borne spores, about 5-15μ in average size, can spread over a wide area and contaminate food, cause diseases for both plants and animals, and act as allergens for humans.

Similar to bacteria, the cytoplasmic membrane of a fungus is surrounded by a cell wall structure. Unlike bacteria, the fungal cell walls are lamellar with each layer consisting of fibrils criss-crossing one another in various directions.[50] The principal constituents of the fungal cell wall are various types of polysaccharides with minor amounts of lipid and protein. Chitin has been reported as the common polysaccharide component present in all fungal cell walls.[51]

Fungi are known to survive in severely stressful conditions where bacteria cannot. Due to their tougher cell wall structure, fungi can withstand high osmotic pressures. Fungi can also tolerate a wide range of pH and can extract water from solutions of high salt or sugar concentration when necessary to support growth. Although fungal diseases are not as widespread as those caused by bacteria, they are harder to diagnose clinically. Fungal diseases usually progress more slowly and pose a chronic health problem. Since both fungi and humans are of eucaryotic cell type, any agent that kills fungal cells may also cause damage to the human body. This is the reason that while many antibiotics have been developed to overcome infections caused by procaryotic cells, very few treatments have been developed to target fungi effectively.

Cancer Cells

So far, the discussion has focused on normal cellular structures. In humans and other multicellular organisms, the volumes of the individual tissues and organs are relatively constant and appropriately proportioned according to the size of the body. Furthermore, the organization of the tissues within organs and the differentiated character of individual cells of the tissues are also stable. This stability of tissue volume, differentiation, and organizations is particularly important to the maintenance of normal function. However, in a multicellular organism the regulation mechanisms controlling cell division sometimes may go wrong. In this situation, the stability of the organization of tissues and organs is destroyed. Consequently, a variety of diseases arise. One example is the formation of tumor due to uncontrolled growth. Tumors can be classified either as benign or malignant based on their tendency to spread. The common term for a malignant tumor is cancer. Cancer is a disease originating from abnormal gene expression.

Cancer cells develop from a single progenitor cell that has undergone a series of permanent, heritable changes. This process, called neoplastic transformation, involves a number of mechanisms and includes direct damage to DNA (such as gene mutations, translocations, or amplifications) and abnormal gene transcription or translation.[52] Cancers arising from epithelial structures are called carcinomas; those that originate from connective tissues, muscle, cartilage, fat or bone are called sarcomas; and malignant tumors affecting hemopoietic structures, including the immune system, are called leukemias and lymphomas.

Often, cells from either animal or human origin can be cultured *in vitro* independent of other cells. This kind of tissue culture has become the most useful means to study the regulation of tissue growth in both normal and cancerous cells. In a cell culture normal cells are able to undergo only a finite number of cell doublings before they lose the ability to divide. However, cancer cells lack this regulation and are essentially immortal, capable of undergoing unlimited numbers of division. For example, the first cancer cells successfully brought into culture were isolated originally from cervical carcinoma in 1951. These cells were named Hela cells, in honor of Henrietta Lacks, the woman who contracted the cervical carcinoma and eventually died of the disease. These cells have been in culture for more than 40 years and have undergone countless cell doublings with no sign of diminishing vigor. Hela cells still grow vigorously and are used worldwide. Normal cells grown in cultures typically resemble the cells from which they are derived and tend to retain some degree of differentiation that sets them apart from cells isolated from other tissues. However, cancer cells grown in culture will often look completely different from the tissue from which they are derived. Certain cancer cells retain no differentiating features at all, making it impossible to determine their origin.

Biological Effects of TiO_2 and Photocatalytic Chemistry

The first report of photocatalytic disinfection was the work of Matsunaga and coworkers in 1985.[1] That work and the research that has followed have been primarily with bacteria and tumor cells but there are a few studies on yeasts, viruses, and other types of cells. *E. coli* has been the most studied organism. Most work has been in aqueous phase, but there are reports of removal of bacteria from humid air.[53,54,55] Table IV summarizes the organisms that have been studied, some of their characteristics, and references to the work on photocatalytic responses. Representative conditions and results are presented in Table V. The work done and results are discussed in the following sections.

Table IV. Organisms Tested with Titanium Dioxide Under Light Conditions

Organism	Gram +/-	Shape/Size (μm)	Reference
Bacteria			
Escherichia coli	-	rod/1 X 3	1, 25, 56, 58, 59, 66, 68, 69, 71, 72, 77, 78, 80, 81, 82, 87, 90, 172, 174
Pseudomonas stutzeri	-	rod/ 0.5-1 X 1.5-4	84
Seratia marcescens	-	rod/0.5-0.8 X 0.9-2.0	53, 54, 56, 66
Staphyloccus aureus	+	spherical/0.5-2.0	66, 77
Clostridium perfringens spores		oval, subterminal	87
Salmonella typhimurium	-	rod/0.7-1.5 X 2.0 - 5.0	25, 169
Streptococcus mutans	+	spherical/0.5-2.0	67, 94, 70
Lactobacillus acidophilus	+	rod/0.6-0.9 X 1.5-6.0	1
Streptococcus cricetus	+	spherical/ < 2	76
Streptococcus rattus	+	spherical/ <2	76
Actinomyces viscosus	+	rod/0.5 X 1.6-2.0	76
Bascillus pumilus	+	rod/0.6 X 2-3	83, 86
Streptococcus sobrinus	+	spherical/ < 2	73
Bacillus subtilis	+	rod/0.7-0.8 X 2-3	77
Yeast, Fungi			
Saccharomyces cerevisiae		oval/3.5-7.0 X 3.5-9.0	1, 71
Candida albicans		oval/~7	76
Hyphomonas polymorpha		multiple shapes	68
Cancer Cells			
HeLa		14-16	63, 64, 100, 101, 102, 103, 108

(continued)

Table IV. Continued

Organism	Gram +/-	Shape/Size (μm)	Reference
T24		30	109, 110, 112
U937		14-20	113
Mouse lymphoma L5178Y		11-12	169
Viruses			
Phage Qβ		0.028	116
Phage MS-2		0.024	74, 75
Poliovirus 1		0.028	57
Lactobacillus phage PL-1		0.05	115
Other			
Human skin fibroblasts		16-18	121
Alveolar macrophage		20-30	135
Chinese hamster CHL/IU cells		14-16	169

Bacteria, Fungi, and Yeasts

Most studies have been done with organisms grown in laboratory cultures with the exception of work on pond water[56] or secondary waste treatment effluent.[56,57,58,59] The usual procedure has been to grow the organisms, separate them from the culture medium, wash them, and then resuspend them to a known concentration in buffer, saline solution, or deionized water. The same method has been used in the studies of disinfection of air. In the air phase work the cell suspension is converted to an aerosol by use of an atomizer or nebulizer.[54,55] The nature of the salt content of the liquid medium influences the rate of disinfection in ways similar to effects on reactions of simple organic compounds in water. It has been found that in general salts inhibit reaction.[6,11,60,61] Phosphate exhibits the greatest effect and chloride the least. Phosphate has also been found to inhibit the adsorption of basic amino acids on TiO_2.[62] Carbonate and other species that can react with hydroxyl radical in competition with the target species also interfere and reduce the efficiency and observed rate of reaction.[6,32] Residual thiosulfate used to remove

Table V. Representative Conditions for Photocatalytic Experiments

Organism	pfu or cfu/mL /Medium	TiO_2 Type/Loading	Light Source/Output	Reactor	Kill Fraction	Time (min)	Comment	Reference
Polio virus 1	10^3/secondary wastewater effluent	Anatase/250 mg/L	A)Sunlight B)F40BL/2 suns	15 cm diameter dishes	>99% 99	30 30	No differences in the pH range of 5-8.	57
Phage MS2	10^4/phosphate buffer	P25/1g/L	J-205, 15W BL/ 2 mW/cm^2	1 L beaker containing 100 mL	99.9 with $FeSO_4$ 90 without	10 10	This was the first virus killing study. 55 min stirring with TiO_2 in dark had reduced the counts from $6x10^4$ to 10^3 before the light was turned on.	74,75
Lactobacillus Phage PL-1	10^7/phosphate buffer with 0.0003% gelatin	Cleansand-205	36 W fluorescent lamp Toshiba FLR-40S	Test tubes in a water bath	>99	200 day	Cleansand is silica sand coated with a mixture of SiO_2, Al_2O_3, TiO_2, and Ag sintered at 200 °C.	115
Phage QB	10^5/DI water	Anatase/0.1 g/L	FL20S-BLB and GL20 3 mW/cm^2, from top	A) Slurry in petri dish B) Suspension on TiO_2 coated tile	99.6 99.4	20 60	The TiO_2 coated tile (Toto Ltd) was developed for interior sterilization.	116
Escherichia coli	35/treated municipal waste water	BDH-GPR/ 2 g/L	T10 20W BL/45 W/m^2 internally	Annular reactor	98	60	Nearly same removal with light and aeration only as with TiO_2 present, dose model and light flux effects given.	79
	10^3/phosphate buffer	P25/ 0.4 mg/mL	Xenon 300W, 60 mE/sec/m^2	Optical fiber reactor	100	120	Survival over 40% for >106 cells/mL. Diffuse and conventional optical fibers compared	82
	$3.1x10^5$/dechlorinated tap water	TiO_2 on fiber glass mesh	UV lamp (300-400 nm)	Annular reactor, 4 tubes, (Nutech Environ. Inc.)	100	9	No reduction when $S_2O_3^{-2}$ was present (due to competition for holes).	56
	10^6/DI water	P25/0.1 to 1 g/L	Two F40CW plus DEK Sylvania projector lamp on either side	175x22 mm Pyrex tube in thermostatic aquarium bath	100	30	No killing with N_2 flow rate proportional to $(TiO_2$ dose$)^{1/2}$ and killing directly proportional to light flux.	69
	10^6/DI water	P-25/ 0.1 g/L	Sunlight	Petri dishes	100	30	100% killing in dark due to temperature rise.	
	10^6/DI water	P-25/ 0.1 g/L	A)Two F40CW plus DEK Sylvania projector lamp 1.7 mE/sec/m^2 B)15 W Phillips fluorescent lamp/0.270 mE/sec/m^2 internally	A) 175x22 Pyrex tube in aquarium bath with ultrasonic tip B) Recirculating batch photoreactor with ultrasonic tip in reservoir	~100 5 with son 80 without	120 120 120	20% killing by ultrasound alone.	68
	10^3/drinking water	n-type TiO2/1 g/mL	3 Phillips TL8/08BLB	Not specified	100	60		80

(continued)

Table V. Continued

Organism	pfu or cfu/mL /Medium	TiO_2 Type/Loading	Light Source/Output	Reactor	Kill Fraction	Time (min)	Comment	Reference
	10^2-10^5/DI water	P25/1 mg/mL	100 W Hg/1,100 mE/sec/m^2 externally	A) Slurry B) 26 glass tubes (30 cm x 6.5 mm) containing TiO_2 membrane continuous flow	16-100 64-100	30 16	Killing depended on the total load of TiO_2, max for 4.8 gram in the system. Killing depended on the number of cells, suggest mechanism involving oxidation of Coenzyme A.	85
	10^3/phosphate buffer	P25(10%Pt)/2.5 g/L	500 W white fluorescent 400 W metal halide 4.6 mE/sec/m^2 from top	Slurry	80	60	This was the first bactericide study.	1
	10^3/DI water	P25/0.1 mg/mL	Sunlight 32 black lights, 50 W, from to	Stirred pans (250 mL) Slurry in petri dishes	100 100	20 7	More rapid kill was obtained by stirring.	66
	10^3/surface water	P25/ 1 mg/mL	3x8W BL fluorescent lamps 5.6 mW/cm^2, from bottom	Glass vessels, 7.5x3.5 cm	100	60	The effect of TiO_2 loading determined.	81
	10^4/DI water	not specified/0.1 g/L	Sunlight	250 mL screw cap flasks	99	23	Temperature 20 ^{0}C.	72
	10^5/DI water	NTi-500	FL15 BL-B/1 mW/cm^2 from top	Liquid film on TiO_2-coated glass plate	100	60	High survival ratio in a separated system using a porous PTFE membrane (50 mm thickness).	174
	10^9/saline	P25/0.01-0.5 g/L	500 W high-pressure mercury lamp/0-60 W/m^2, externally	Rectangular bubble-column (40x40x250 mm) solution height 170 mm	100	4 to 12	Sterilization rate constant was determined on a series-event model and a second-order kinetics.	71
E. coli plus	3x10^3/DI water	TiO_2 film on titanium	Two 8 W UV-B lamps	Photoelectrochemical	100	25	No effect of potential difference alone.	87
C. perfringens	6x10^3	metal electrode		cell with nickel mesh counter electrode and Ag/AgCl reference	99	25		
Total coliforms	5x10^7/seconday waste water effluent	Anatase/0.25 g/L	F40BL/2 suns, from top	15 cm diameter dishes	99	150	Rate model and constants given.	57
Fecal coliforms	10^6/same				99	120		
Total coliforms	secondary waste water	Eastman Kodak	50 W low pressure mercury 30 mW-sec/cm^2 internally	Stainless steel annular chamber coated with TiO_2 30.5 cm in length with 5.1 - 10.2 cm interior diameter	< 10	0.2	Need to increase surface area to volume ratio to improve the disinfection.	58
S. marcescens	10^4/ phosphate buffer	P25/ 0.1 g/L	32 black lights, 50 W	Petri dishes	100	8	Phosphate, carbonate, and amino acids inhibit killing effect.	66
S. marcescens	10^9/spent broth (initial cell density 400 cfu)	P-25 on fiber glass filter	24 RPR-3500A lamps ~36 W output, internally	Recirculating air loop	100	500	~80% deactivation by light alone at 50% relative humidity, no TiO_2 effect at 30%, less inactivation at 85% relative humidity	53 55

Organism	pfu or cfu/mL /Medium	TiO_2 Type/Loading	Light Source/Output	Reactor	Kill Fraction	Time (min)	Comment	Reference
P. stutzeri	$5X10^6$/Ringer solution	Tioxide NP92/0.5 g/L	18 W BLB, TLD18W/08 8.1 W/m^2, from top	Slurry in Petri dish on shaker	100	240	Killing rate increased with increasing TiO_2 concentration.	84
	not specified	not specified	Source not specified 2 W/m^2	Thin film, generated as a "water bell"	>99	30	Demonstrates the use of a novel reactor for contacting a thin film with light.	89
B. pumilus spores	10^9/DI water	Anatase/2 mg/mL	Blak -Ray, 22 W/m^2 from top	Slurry in Petri dish (60x15 mm) on shaker	95	120	Max. killing for 109 cfu/mL, increased killing when 15 min cycles of light and dark were used compared to cont. light.	83
S. aureus	10^4/phosphate buffer	P25/ 0.1 g/L	32 black lights, 50 W	Petri dishes	100	10		66
L. acidophilus	10^3/phosphate buffer	P25(10%Pt)/2.5 g/L	500 W white fluorescent 400 W metal halide	Slurry	100	60		1
S. sobrinus	10^5/saline	P25, 1 mg/mL	Two FL20S-BL	Stirred vial, 3x5 cm	100	1	Co-aggregation observed $>10^5$ cfu.	73
	10^9/saline				100	90	co-aggregates > 1 mm observed, Potassium, protein, and RNA release. Results similar to *S. sobrinus*. No differences in the bactericidal effects between the 7 strains covering all serotypes	
S. cricetus	10^4/broth plus saline	Rutile/1 g/L	Fluorescent, 578 nm	Aerobic growth chamber	50	60	Equal effect in light or dark.	76
S. rattus FA-1					100	60		
S. rattus BHT					0		No effect in light or dark.	
A. viscosus					100	15		
S. cerevisiae	10^3/phosphate buffer	P25/0.25 g/L	400 W metal halide	Slurry	80	120	88-91% survival in dark conditions.	1
		P25(10%Pt)/0.25 g/L	4600 mE/sec/m^2 from top		100	120	46% killing in 60 min.	
C. vulgaris	10^3/phosphate buffer	P25(10%Pt)/0.25 g/L	400 W metal halide 4600 mE/sec/m^2	Slurry	15	60		1
C. albicans	10^4/broth plus saline	Rutile/1 g/L	Fluorescent, 578 nm	Aerobic growth chamber	0		No effect in light or dark.	76
Hela cells	10^5/CMF-PBS	Colloid TiO_2 (P25)	500 W high pressure Hg	TiO_2 thin film on SnO_2 electrode connected to a potentiostat (+0.5V)	100	10	This is the first cancer cell killing study.	64
	24 hrs culture/MEM	P25/12-120 mg/mL	Super-high pressure mercury lamp	Slurry	100	10 to 30	Cells were pre-incubated with TiO_2 for 24 h.	100

(continued)

Table V. Continued

Organism	pfu or cfu/mL /Medium	TiO_2 Type/Loading	Light Source/Output	Reactor	Kill Fraction	Time (min)	Comment	Reference
	24 hrs culture/MEM	P25/120 mg/mL (0.03-10 nm)	500 W high pressure Hg	TiO_2-free MEM	100	25	TiO_2 may be absorbed into the cytoplasm. Ultrafine TiO_2 particles were found on cell membrane and inside cells.	102
T-24 cell	10^4/PBS	TiO_2 microelectrode (~10 mm in diameter)	150 W Hg-Xe, filtered	TiO_2 microelectrode (+1.5 V) on fixed single cell	100	3	The TiO_2 microelectrode has to be in contact with cell.	112
U937 cells	10^6/PRMI 1640	Colloid TiO2/1 mg/m (10 nm)	500 W high pressure Hg filtered	Slurry	100	30	U937 cells have been pre-incubated with TiO_2 for 2 h.	113

chlorine from a reactor being cleaned prior to use was found to inhibit the photocatalytic killing of *E. coli*.[56] The use of deionized water for the cell medium eliminates the interference of anions and organic compounds on the photocatalytic chemistry. However, prolonged suspension of cells in deionized water can result in weakening of the cell walls due to loss of calcium and magnesium ions from the surface. The weakened cell wall is less able to resist the internal osmotic pressure that results from the solute concentration difference between the cell and the water. This can make the cell wall more permeable than normal or result in damage that weakens or kills the cells.

The choice of light source and reactor configuration has been highly variable. In general, experiments have shown that titanium dioxide suspended in water has no significant effect on survival of bacteria in the dark, and the effect of light plus titanium dioxide is greater than the effect of light alone. However, with some light sources there is significant reduction in colony forming units (cfus) with light alone. This is expected to occur particularly with lamps that have output below 300 nm and when the optical components of the reaction vessel will transmit the shorter wavelengths.[58,63,64] One study compared two lamps each with a photon flux at wavelengths below 380 nm of about 400 mE/hr. The lamp with about 41% of its output below 315 nm showed greater killing both with and without TiO_2 than the lamp with only 4% of its output in that range.[59] Another report found germicidal light more effective than black light.[65] Regardless of the type of light source used, the number of photons delivered (on the order of $10^{-3}E/m^2sec$) is orders of magnitude greater than the number of cells in a typical experiment (in the range of 10^3 to 10^9/ml). Experiments using light sources with fluxes in the range of about 100-2000 $\mu E/m^2s$ caused 100% deactivation of *E. coli* in times ranging from 7-120 min. A reactor of commercial design using four 40 W lamp reactors in series (unspecified spectral output) achieved 99.999% deactivation within 9 minutes.[56] Experiments with sunlight produced comparable results.[66]

Fluorescent black lights with an emission maximum at about 365 nm have been the most commonly used since there is a good match with the band gap of anatase. Medium pressure mercury arc and xenon arc lamps have also been used with a Pyrex™ filter to eliminate the UV below about 290 nm. Some studies have been done using cool white fluorescent lamps, with a peak output at about 578 nm,[67,68,69] metal halide lamps,[1] and projector lamps.[69] The emission

spectral properties of the lamps and photon fluxes at the reactor have not always been provided in the published reports which makes comparison of results difficult. It is not clear how much overlap there is in the action spectrum of titanium dioxide with the output of some of the lamps used, e.g. cool white fluorescent. Comparable killing of *S. mutans* was reported for two types of TiO_2 under UV and white light.[70] An early study compared the effect on *S. cerevisiae* of metal halide, xenon, and white fluorescent lamps with the same photon flux using platinized P25 TiO_2 as the photocatalyst. The surviving fractions were 27%, 46% and 58% respectively.[1] A series event model has been developed which features second order kinetics with respect to the concentrations of microbial cells and oxidative radicals to predict the survival of *E. coli* and *Saccharomyces servisiae* in aqueous slurry with irradiated titanium dioxide. The model was originally developed in the context of chlorine and chloramine disinfection of water in which the death of a cell was caused by a number of reactions, n, between oxidative species and the cell. The predictions of sterilization rate as a function of catalyst concentration and light intensity are fairly accurate, particularly given the use of a theoretical rather than empirical mathematical model.[71]

Sunlight has been demonstrated to have sufficient light in the near UV region (below 400nm) to activate TiO_2.[10,72] This has been exploited for disinfection applications. In solar experiments where the temperature was not controlled, deactivation was the same with and without TiO_2 due to the temperature rise.[66,69]

Some workers have reported that there is a modest decline in colony forming units when the cell suspension is added to titanium dioxide. This has been attributed to agglomerization of cells and TiO_2 particles and removal of cells by sedimentation. This effect was investigated in some detail for *S. sobrinus* where it was found that the size of aggregates depended on both the concentration of cfus and TiO_2 (P25). Aggregates larger than 1 mm were observed for TiO_2 loadings over 0.1 mg/mL and $>10^8$ cfu/mL.[73] A similar effect was observed with a virus.[74,75]

Most investigations have used the anatase form of titanium dioxide as the photocatalyst, with Degussa P25 being the most commonly used. Rutile has also been reported to be active for the killing of *S. cricetus,* but not *S. rattus,* with a fluorescent lamp having a maximum output at 578 nm.[76] Platinum on rutile enhanced the killing of *E. coli* and *S. aureus*.[77] Ten percent platinum on TiO_2 (P25) enhanced the killing of *S. cerevisiae* from 80% to 100% in a reaction time of 120 min.[1] Silver on TiO_2 was found to be more effective than TiO_2 alone or TiO_2 with platinum for the killing of *E. coli*.[78]

The effect of TiO_2 particulate loading in suspensions has yielded mixed results. Destruction of *E. coli* and total coliforms reached a maximum at a TiO_2 concentration of 2 mg/mL then declined with further increased loading in an annular reactor.[79] An optimum loading of 1 mg/ml of P25 was reported for deactivation of *E. coli* in presterilized surface water using a black light in a stirred reactor.[80,81] In a reactor with conventional optical fibers as the light source and emitting only at the tips, maximum killing of *E. coli* was observed at 0.0025 mg/ml and the rate declined rapidly up to 0.25 mg/mL. The effect was attributed to shading by the suspended solids. For fibers modified to emit axially from the body as well as the tips, the maximum killing did not decrease significantly between 0.0025 and 0.25 mg/mL. This was attributed to better distribution of the photons through the volume of the reaction mixture.[82] In another study, killing of *E. coli* declined significantly up to about 0.2 mg/mL and continued to decline, but less rapidly, up to about 1 mg/mL.[69] For *S. marsescans* the optimum loading was found to be 0.1 mg/mL. For coliforms using a SR-2 lamp, the maximum killing was observed with a loading of 0.04 mg/mL (this could be due to shading if the killing was predominantly due to the short wavelengths).[58] For *B. pomilus* spores an optimum loading of TiO_2 was found to be about 2 mg/mL.[83] For *P. stutzeri* the greatest decrease in viable cell count was obtained using 4 mg/ml of TiO_2 (Tioxide NP92).[84]

The dependence of the rate of cell killing as a function of initial concentration has been variable in the studies reported to date. The reaction rates have often been fit with first order kinetics. This was the case for total coliforms.[57,79] In the range up to about 10^3 cfu/mL, the rate is usually independent of initial cell concentration.[79] As cell count increased from 10^2 to 10^5 cfu/mL percent survival went from zero to 84%.[85] For *B. pumilus* spores cell killing increased for initial cell concentrations between 10^4 and 10^9 cfu/mL then declined for 10^{10} cfu/mL.[83,86] It is not known whether there is a connection between the decreased efficiency at high cell counts and the observation that at high cell loadings co-agglomerization with TiO_2 can occur.

Alternating 15 min periods of light and dark was reported to enhance the killing of *B. pumilus* spores.[83] The combination of light and ultrasound was found to enhance the rate of killing *E. coli* with suspended TiO_2.[68] The removal of *E. coli* and *C. perfringens* spores was significantly enhanced when a positive voltage bias was applied to an irradiated TiO_2 coated electrode.[87] *E. coli* were deactivated on films of TiO_2 coated on tiles.[88] A novel reactor based on a "water bell" has been modelled. It has the potential to give efficient use of light and avoids contact of

the light source and water.[89] With TiO_2 immobilized in acetyl cellulose >99% deactivation was maintained with water containing 10^2 cfu/ml flowing for one week at residence time of 16 min.[85]

Evidence for the mineralization of *E. coli* cells on a photocatalytic surface exposed to air and light has been recently reported.[90]

Killing of total coliforms in diluted secondary waste effluent containing 50-80 cfu/mL was found to be enhanced by a small amount with added TiO_2 when a commercial reactor with low pressure mercury arc lamps was used.[58] For coliform counts of $5X10^7$ cfu/mL, 150 min was required to achieve 99% deactivation with a 40 W black light.[57] Total coliforms in pond water samples were reduced by >99% while the heterotrophic plate count only decreased by about 10%.[56] The use of a black light reduced the number of coliforms and bacteria was reduced from 35,000 to 59 per 100ml in 60 min.[79]

The susceptibility of four kinds of organisms to killing by the photocatalytic effect was compared using platinized P25 TiO_2 and a metal halide lamp. *L. Acidophilus* (Gram +), *E. coli* (Gram -), *S. Cerevisiae* (yeast), and *Cl. vulgaris* (algae) were deactivated to the extent of 100%, 20%, 54%, and 45%. The first three were accomplished in 60 min and the last required 120 min. This correlates roughly with the thickness of the cell wall.[1]

Dental Applications

The mouth is home to a large and very diverse population of microbes comprising over 350 taxa, including at least 37 genera of bacteria.[91] Cell to cell co-aggregation has been confirmed with isolates from 18 genera, constituting those bacteria most commonly identified in dental plaque. Essentially all oral bacteria possess surface molecules that show some sort of cell-to-cell interaction. The earliest colonizers are overwhelmingly *streptococci*, which constitute 47 to 85% of the culturable cells found during the first 4 h after professional cleaning of teeth.[92] Within 12 h the population diversifies to include *actinomyces*, *capnocytophagae*, *haemophili*, *provotellae*, *propionibacteria,* and *veillonellae*. Some cariogenic mutant *streptococci* synthesize extracellular glucans and a surface protein that contribute to their ability to adhere to teeth.[93] Generally, the ability to attach to bacteria already anchored to hard or soft tissue may provide secondary colonizers. The oral bacterial adherence poses a great challenge for dental hygiene. A number of

studies have been done on the effect of photocatalytic treatment on bacteria found in the mouth. These include *S. sobrinus*, *S. mutans*, *S. rattus*, *S. cricetus*, *C. albicans*, and *A. viscosus*.[67,70,73,76,94] Titanium dioxide added to pit and fissure sealants was found to inhibit adherence by *S. mutans* when irradiated with a fluorescent lamp (peak output at 578 nm) in saline solution.[67] Titanium metal was found to be one of the most active of the dental implant metals in *in vivo* antibacterial tests on *P. endodontalis*, *P. gingivalis*, *P. intermedia*, *P. melaniogenica*, *A. actinomycetemcomitans*, *A. naeslundii*, and *A. viscosus*.[95]

After a few weeks a broad spectrum of microbes can be found on a new toothbrush.[96] In a recent pilot study using a range of selective growth media, the total microbial load per toothbrush was found to be 10^4 to 10^6 cfu. *Staphylococci* and *streptococci* were dominant. *Candida*, *corynebacteria*, *pseudomonas*, and coliforms were also identified.[97] Light activated tooth brushes based on photocatalytic process have been tested and found to control dental plaque.[98,99]

Tumor Cells

HeLa cells (cervical carcinoma) were killed by exposure to light from an unfiltered 500 W mercury arc lamp in the presence of TiO_2 (P25). Cells were cultured in Minimum Essential Medium (MEM) in the presence of TiO_2 for 24 hrs, then exposed to light from a high pressure mercury lamp. HeLa cells were killed even after the external TiO_2 was removed by washing, indicating that TiO_2 particles had adsorbed onto the cell surface or that they were ingested by the cells. The rate of cell killing is increased with greater levels of TiO_2 loading, in the range 0-120 μg/mL.[100] To test the effect of SOD, HeLa cells were cultured as above, and during the final three hours, cells were washed and phosphate buffered saline (PBS) containing SOD was added. Irradiation with a 500 W high pressure mercury lamp filtered to transmit 300-400 nm light resulted in increased killing compared to the case without SOD. The increased killing was postulated to result from hydrogen peroxide formed from superoxide produced at the catalyst surface. An approximate ten-fold increase in hydrogen peroxide concentration was found in the presence of SOD. When catalase was added, the effect of SOD was suppressed. This can be explained by the catalytic decomposition of hydrogen peroxide by catalase.[101] The cell killing effect was more pronounced in the PBS medium than in MEM, possibly due to an internal filter effect or the scavenging of hydroxyl radicals by some of the components of the solution. The hydroxyl radical scavengers mannitol and L-tryptophan reduced cell killing.[100] TEM showed that

TiO_2 particles had adsorbed both on the surface of the cells and had been absorbed into the cells. Tumors caused by transplanting HeLa cells into nude mice were suppressed by irradiation with 300-400 nm light in the presence of TiO_2.[62,63,64, 102,103] This work has been discussed in reviews of applications of photocatalytic chemistry.[104,105,106,107] A method for treatment of tumors based on this approach has been proposed.[108]

The effect of TiO_2 and light on calcium ion uptake from buffer solution by irradiated T-24 cells (bladder transitional cell carcinoma) was monitored. Cells cultured in Ham's F12 medium were exposed to an F12 solution of TiO_2 (P25) for 24 hrs. This resulted in incorporation of TiO_2 particles into the cytoplasm. The cells were exposed to 340 and 380 nm light from a 150 W xenon lamp. Calcium concentration in the cells was monitored by microfluorometry. It was proposed that cell damage occurred in three stages: 1) some cell damage occurs, the membrane becomes somewhat permeable, and calcium concentration in the cell increases, 2) a steady state is reached but cells are still viable, and 3) the cell membrane becomes permeable, calcium concentration increases further, and the cell functions are damaged to the point that the cells die. The time course of the diffusion of calcium into the cell was followed. The observed effects were faster when cells were exposed to 100 μg/ml TiO_2 than for 10 μg/ml.[109,110]

A similar effect of TiO_2 loading on the rate of killing T-24 cells was reported as part of a comparison of cell killing in cultures, tumors, and using a photo-excited titanium dioxide electrode. Tumor growth following the transplanting of T-24 cells into nude mice was delayed up to 30 days using treatment with TiO_2 particles and light. Growth of cultures on thin film titanium dioxide electrodes allowed determination of cell killing as a function of applied potential during irradiation. Increasing cell death occurred as the potential was raised from 0 to +1.0 V.[111]

A TiO_2 microelectrode was used to inactivate a single T-24 cell under UV illumination when the electrode and cell were in contact. The cell was not killed when the electrode was 10 μm from the cell surface. Cells were killed when the potential was more positive than 0.0 V vs SCE. Cells not in direct contact with the electrode were not affected. These results support the involvement of reactive oxygen species such as hydroxyl radicals that have short lifetimes, hence short diffusion distances.[112]

In a more recent study, human U937 monocytic leukemia cells were treated with 1 mg/ml colloidal TiO_2 (10 nm) in RPMI 1640 medium for 2 h at 37 °C followed by irradiation with UV

light (300-400 nm). About 80% of cells were killed after 10 min of illumination and complete killing was obtained after 30 min. TiO_2 treated cells showed the evidence of membrane blebbing and DNA fragmentation, especially the formation of DNA ladder. All of these effects are characteristics of apoptosis. Apoptosis, also known as programmed cell death, is a different mechanism of cell death from necrosis. ROS such as $HO^{\cdot}$, $HO_2^{\cdot}$ and H_2O_2 are proposed to be responsible for apoptosis.[113] It has been suggested that TiO_2 and other mineral particles can induce alteration in protein glycosylation in differentiated U937 cells.[114]

Viruses

Polio virus 1 was 99.9% killed in secondary waste effluent after 30 min of irradiation with a 40 W black light.[57] Phage MS2 was 90 % destroyed in phosphate buffer with TiO_2 (P25) and a 15 W black light. The killing increased to 99.9% when ferrous sulfate was added. This suggested that Fenton chemistry might augment the photocatalytic effect.[74,75] *Lactobacillus* phage PL-1 was deactivated at a slightly greater rate than in the dark when illuminated with a Toshiba FLR-40S 36 W lamp in the presence of Cleansand-205. The Cleansand-205 is a formulation prepared by coating silica sand with a mixture of SiO_2, Al_2O_3, TiO_2, and silver in the ratio of 77.1:22.9:3.3:1.7(by weight).[115] Phage Qβ was deactivated on TiO_2 coated tiles using a black light source. Experiments in an aqueous slurry reactor showed a linear dependence on light intensity in the range 3 to $8X10^{-3}$ W/cm^2. When a germicidal light was used, no difference was found with or without TiO_2 coating on tiles.[116]

Photocatalytic Damage to Cellular Molecules

Photocatalytic oxidation of a very wide range of organic compounds has been observed. Therefore, it is not surprising that cellular molecules, such as carbohydrates, lipids, proteins and nucleic acids can be damaged and subsequently lead to cell death. TiO_2 has shown a pronounced activity in the adsorption of basic L-amino acids such as L-lysine and L-arginine in an aqueous solution.[62] TiO_2 is also capable of absorbing and inactivating various bacteriocin from culture supernatant.[117] It has been demonstrated that the nitrogen moiety in various amino acids are converted predominantly into NH_3 upon exposure to 2 mg/ml TiO_2 (P25) illuminated with a 75 W Hg-lamp.[118] TiO_2 photodegradation of DNA and RNA bases was also confirmed by formation of nitrate, carbon dioxide, and ammonia.[118] Synthetic supercoiled plasmid DNA has

been used to demonstrate free radical activity at TiO_2 surfaces. Both ultrafine and normal size TiO_2 particles can cause plasmid DNA breakage at the concentrations range 0.05 - 0.15 mg/mL after incubation for 8 h at 37 °C. Other environmental particles (PM-10) and amphibole asbestos were also found to be highly reactive for formation of free radicals. It was not specified whether the evaluations were done with the exclusion of ambient light.[119] Such DNA damage is more effective with UV illumination. Direct DNA strand break was seen with 0.0125% of TiO_2 after illuminating with simulated sunlight for 10 min. The same experiment has shown that anatase is more active than rutile.[120] Evidently, DNA and RNA damage caused by TiO_2 oxidation is not confined to strand breakage. Hydroxylation of guanine has been demonstrated in calf thymus DNA. The formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in DNA is proportional to increases in both the light intensity and TiO_2 concentration.[121] DNA in normal human cells and cancer cells can also be damaged by illuminated TiO_2.[113,120,121] These findings are consistent with the endocytosis of TiO_2.[102] Cellular RNA has been found to be more susceptible to oxidative damage induced by TiO_2 than DNA. One possible explanation is the compartmentalization of DNA within the cell nucleus.[121] Evidence for the adsorption and subsequent uptake of TiO_2 particles by cells was reported for lung macrophages.[122]

Toxicity Studies of TiO_2 Particles

Titanium dioxide has generally been regarded as a nuisance dust in man.[123,124] The first epidemiologic survey of respiratory disease among 209 titanium metal production workers showed that 17% of the subjects had signs of pleural disease which suggests that reductions in ventilation capacity may be associated with the exposure to titanium tetrachloride and titanium dioxide.[125] However, the epidemiologic study of 1576 workers exposed to TiO_2 particles shows no statistically significant association between TiO_2 exposure and risk of lung cancer and chronic respiratory diseases. No cases of pulmonary fibrosis has been found among TiO_2 exposed workers.[126] The same conclusion was reached in another epidemiologic study of 2477 employees from TiO_2 plants that showed no statistically significant association between titanium tetrachloride exposure and risk of lung cancer and chronic respiratory diseases.[127] However, some pathologic changes such as pulmonary fibrosis and skin necrosis may be associated with direct exposure to large quantities of TiO_2 particles.[128] It is not clear whether the effect of ultraviolet or visible light is involved in these cases.

Mice receiving a saline suspension of 25 mg TiO_2 intraperitoneally showed no foreign-body reaction or tumorigenesis.[129] Those receiving three daily injections show a weak positive on bone marrow micronocleus assay.[130] A feeding study has shown that TiO_2 coated mica produced neither toxicological nor carcinogenic effect in rats at dietary concentrations as high as 5.0% for 130-week.[131] It has also been reported that in macrophage cells at the base of the human gut, associated lymphoid tissue, become loaded early in life with dark granular pigment that is rich in titanium and other mineral particles. These cellular pigments that are partially derived from food additives and partially from the environment, can cause chronic latent granulomatous inflammation.[132]

Studies on transmigration of TiO_2 particles in rats after inhalation have shown that free TiO_2 particles can be retained in the nasal and tracheobronchial epithelium without cellular damage.[133] The uptake of TiO_2 particles by pulmonary epithelial cells is affected by particle size: ultra fine TiO_2 (~20 nm) appear to enter the epithelial cells faster than fine TiO_2 particles (~120 nm).[134] The aggregation of dust-laden macro phages (dust cells) have been found in the lymphoid tissue of the submucosa. Inhaled particles are mostly engulfed by alveolar macro phages and confined to the alveolar duct region.[133] Such phagocytosis phenomena have been demonstrated *in vitro* by flow cytometric assay.[135] Intratracheal instillation of TiO_2 particles (50 mg/kg) in rats can stimulate the release of pulmonary damage factors, e.g., macrophage fibronectin.[136] However, this phenomenon was not seen in inhalation experiments.[137] A fraction of the inhaled particles has also been found in the membranous pneumocytes and interstitial lymphoid. Dust cells in the hyperplastic peribronchial lymphoid tissue are usually eliminated via airways. Transfer to lymph nodes accounts for most of the postexposure clearance for TiO_2.[138] At low concentration (0-50 mg/m^3) no significant pulmonary response has been detected.[132,139] However, inhalation of high concentrations of TiO_2 particles in rats results in impaired pulmonary clearance and persistent inflammation. A decreased pulmonary response has been observed in rats exposed to TiO_2 (125 mg/m^3) for 2 h.[140] High dose exposure to TiO_2 (250 mg/m^3) 6 h/day, 5 day/week for 4 weeks can produce sustained pulmonary inflammation, enhanced proliferation of pulmonary cells, impairment of particle clearance, deficits in macrophage function, and the appearance of macrophage aggregates at sites of particle deposition. [141] Significantly prolonged long-term lung clearance also has been observed in both ultrafine TiO_2 particles and pigmentary TiO_2 particles (250 nm).[142] Free radicals such as hydroxyl radical may be involved in the interactions of TiO_2

particles with pulmonary cells.[143] A few unique types of experimentally induced lung tumors have been observed under exaggerated exposure conditions or long term overload,[144,145,146] but because they are rarely seen in humans, their relevance to humans is questionable. Although initially an irritant, TiO_2-induced pulmonary lesions regressed during a one year period following cessation of exposure.[147] A chronic TiO2 inhalation study of hamsters exposed to 30-40 mg/m^3 for 6 h/day, five days/week, for 18 months has shown chronic inflammatory response.[148] Lung response at 10 mg/m^3 for 6 h/day, 5 days/week for 2 years satisfies the biological criteria for a nuisance dust.[145]

Study on the lungs of mice intratracheally injected with TiO_2 showed no effect on the incidence of lung tumors.[149] However, connective-tissue breakdown has been demonstrated in the rat lung after a single intratracheal instillation of 30 mg rutile TiO_2, indicating that this process probably plays a role in dust-induced emphysema.[150] A similar experiment carried out in dogs suggests that mild lung fibrosis can be induced with large amounts of TiO_2 particles deposited in the lung tissue.[151] Such pathologic change has also been found in a 43-year old male who has been engaged in packing TiO_2 for about 13 years.[152] Formation of DNA adducts following chronic inhalation of TiO_2 particles was not detected in rat lung. Intratracheal instillation of TiO_2 at the range 5 to 100 mg/kg in rats also caused mild increases in the recruitment of inflammatory cells, such as neutrophils, lymphocytes, and alveolar macrophages.[153] Some TiO_2-laden dust cells may enter the peribronchial lymphaticus or pulmonary blood vessels and subsequently migrate into the general circulation.[133] An *in vitro* test has shown that TiO_2 particles can cause the lipid peroxidation of erythrocytes and subsequent haemolytic reaction.[154] Since there were no tissue responses to translocated particles in the lymph nodes, spleen, or liver, potential adverse health effects therefore appear to be negligible. In a rat liver epithelial cell assay, neither ultra fine TiO_2 particles nor pigmentary TiO_2 particles have direct clastogenic potential.[155] The genotoxicity of TiO_2 remains to be controversial. The micronucleus test *in vitro* using Chinese hamster ovary cells shows that TiO_2 did not induce micronuclei due to poor solubility in the culture medium.[156] However, it has also been demonstrated that TiO_2 can be transported into hamster ovary cells and exhibit potential genotoxicity.[157]

TiO_2 has been noted to be a safe physical sunscreen. Animal tests have shown that micro fine TiO_2 completely protects mice from UV-induced carcinogenesis[158] and protects the skin's immune system.[159] Such protections are significantly affected by the application thickness.[160]

TiO_2 also shows a satisfactory protective capacity in those photodermatoses.[161] Recently, the percutaneous absorption of titanium in the epidermis and dermis were observed in subjects applying micro fine TiO_2 sunscreens daily for 2-4 weeks.[162] The distribution of TiO_2 within the different layers of human skin has been demonstrated using a pulsed form of the photoacoustic technique.[163] It has been shown that sunlight-illuminated TiO_2 can cause DNA damage.[120] These results may be relevant to the overall effect of TiO_2 in sunscreen and cosmetic products.

Generally, TiO_2 is considered to be a non-pathogenic, inert mineral particle.[139,149,164,165,166,167] Usually, in the absence of UV light neither anatase nor rutile exhibit much biological activity.[168] Pathogenic effects of TiO_2 particles are usually due to the general physical stimulation activity. However, with UV light irradiation TiO_2 particles exhibit significant cytotoxicity and potential photogenotoxicity.[169]

Mechanism of Cell Killing

There are numerous papers dealing with the bactericidal effect of TiO_2 photocatalysts over a wide range of microorganisms, however, only a few publications have investigated the various modes of action TiO_2 exerted on cells which lead to cell death. A fundamental understanding of the underlying principles of the cell killing mechanism is critical in divising feasible disinfection and medical treatment systems.

There are several possible mechanisms for cell killing by the photocatalytic process. The earliest that was proposed was that of Matsunaga, who presented evidence for the oxidation of coenzyme A (CoA) in *S. cereviaiae*, a yeast, when exposed to light and platinized TiO_2.[1] Upon illumination of the cells in the presence of TiO_2/Pt for 120 minutes under a metal halide lamp, more than 97% of the intracellular CoA content was lost as compared to a 42% loss when TiO_2 was omitted. Under the same conditions, the respiratory activity was also decreased to 42% of those of the untreated cells. The authors attributed the loss of intracellular CoA level to be the root cause for the decrease in respiratory activities which ultimately led to cell death. An earlier attempt to identify the oxidized product as dimeric CoA was inconclusive since both the dimer and the cell lysate had similar *Rf* value by thin-layer chromatography on silica gel. Later, when pure CoA was incubated with TiO_2 under light, a stoichiometric increase in dimeric CoA was observed, presumably a sulfur-bridged dimer, with the concomitant loss of CoA (Matsunaga et al., 1988,

#770). Serving as a carrier for the acyl groups, CoA participates in many enzymatic reactions involved in the respiratory chain and fatty acid oxidations.[170] The terminal sulfhydryl group of CoA is the reactive site of this molecule for the acyl transfer reactions, therefore, its photooxidation reaction would be detrimental to cell viability.

The actions of the highly oxidized species generated on the surface of the illuminated TiO_2 are generally regarded as non-selective, therefore, it is reasonable to expect that the cell membrane would have to be oxidized first, losing its semipermeability before the intracellular CoA is photooxidized. Yet, on the basis of either light or electron microscopy, Matsunaga and his coworkers failed to detect any destruction of the cell wall by photo-activated semiconductor powders.[1] When *S. cerevisiae* cells were physically separated from the TiO_2/Pt particles by a dialysis membrane to prevent any direct contact, no loss of either respiratory activity or cell viability was observed. The authors concluded that a direct contact between cells and the semiconductor is a prerequisite for cell killing. Together with the fact that the addition of catalase failed to reduce the killing effect, the authors concluded that oxidative species such as H_2O_2 and free radicals formed during the photocatalytic process were not responsible for the bactericidal effect.

Other workers have found evidence for disruption of the cell wall, cell membrane and leakage of the cell contents.[73] Working with *Streptococcus sobrinus* AHT, Saito and his coworkers discovered a rapid leakage of potassium ions within 3 min upon illumination with TiO_2, and its leakage kinetics coincided with cell death. When high concentrations of K^+ were added back after the reaction, the loss of viability was not reversed. Thus, the loss of K^+ itself is not the direct cause of cell death. Using K^+ leakage merely as a gauge for measuring changes in membrane intactness, the authors concluded that photo-excited TiO_2 had caused a significant disruption of the cell membrane leading to the compromise of its semipermeability, and subsequent cell death. With illumination up to 120 min the much larger molecules such as proteins and RNA were slowly released into the extracellular fractions indicating that major membrane damage had occured. Electron micrographs indicated that many cells were broken open at this stage. The authors detected a drop in the medium pH at this time, probably due to the leakage of the many acidic cellular components and the mineralization of some of them into CO_2. Electron micrographs also indicated that TiO_2 particles did not reach the cell membrane surface until after 30 min of reaction with the peptidoglycan layer, yet death occurred within 1 to

3 min. They proposed that perhaps the ROS were produced under the photocatalytic conditions and these species were able to reach the cell membrane and disrupt its structure directly.

Further evidence of cell membrane disruption by irradiated TiO_2 came from the work of Sakai and coauthors who incubated human malignant cell, T-24,[109,110] with a TiO_2-F12 solution ($[TiO_2]$ = 100 or 10 μg/mL) for 24 hours in the dark. On the basis of the electron micrograph, Sakai and his coworkers found that the TiO_2 particles were distributed not only at the outer surface of the cell membrane, but also in the cytoplasm, probably through the process of phagocytosis, or "cell eating." Phagocytosis is a defense mechanism in which the eucaryotic cell engulfs foreign material holding it in its interior for later digestion.[48] Even with the TiO_2 being incorporated intracellularly, more than 90% of the cells were still viable at this stage. With the onset of light these cells exhibited two-stage Ca^{++} leakage kinetics over 10 min duration of the photocatalytic reaction. After the first 4 min of near UV illumination, a rapid increase of intracellular Ca^{++} concentration was observed while the cells retained their viability. The Ca^{++} level then reached a steady state briefly, followed by a second-stage rapid increase where the viability decreased drastically. The authors attributed the initial elevation as an influx of Ca^{++} through the membrane from outside, indicating that minor cell membrane leakage had occurred. They proposed that the more deadly second-stage leakage was due to the release of Ca^{++} from the internal Ca^{++} stores, such as calcium-binding proteins or organelles as in the endoplasmic reticulum.[171] At this stage, major rupture of the cell membrane and decomposition of essential intracellular components such as organelles had taken place, making cell death unavoidable. The authors attributed the leading cause of cell death to attack of the unsaturated lipid membrane by reactive oxygen species such as hydroxyl radicals, superoxide ions or hydrogen peroxide generated from the photo-excited TiO_2 particles.

Direct evidence of membrane damage comes from the work of Sunada and coworkers using a transparent thin film of TiO_2 to measure the destruction of endotoxin from *E. coli*.[172] The endotoxin is a lipopolysaccharide macromolecule which resides on the outer membrane of Gram-negative bacteria, including *E. coli*.[173] Its toxicity resides mainly on the lipid fraction, i.e., lipid A, while the sugar moiety acts as the antigenic determinant. The endotoxin is an integral part of the bacterial cell envelope and is released only when the intact cellular structure is destroyed. Therefore, the release of endotoxin into the medium is a good indicator for outer membrane destruction. Their results support the concept that TiO_2 photocatalytic reaction causes the

destruction of the outer membrane of the *E. coli* cells, as well as the degradation of toxic compounds subsequently released from the dead cells. By serving both as an antibacterial and a detoxifying agent, the authors concluded that TiO_2 photocatalyst is unique in its environmental applications. Mineralization of whole *E. coli* cells has also been demonstrated recently by Jacoby and co-workers.[90]

To selectively investigate the effect of various reactive oxygen species responsible for the bactericidal effect, Kikuchi and his group incorporated a porous PTFE membrane in their system to physically separate the *E. coli* suspension from the TiO_2 thin.[174] This membrane-separated setup presumed that the less mobile radical species would be unable to traverse the 50 μm distance from the TiO_2 surface to the bacterial suspension while the longer-lived solution phase species would be able topass through. The membrane's pore size of 0.4 μm would also prevent any *E. coli* from migrating to the TiO_2 surface. Excellent photo killing was achieved in their system with and without the PTFE membrane. In both cases, the addition of catalase quenched the killing significantly. The contribution of hydrogen peroxide was obvious as the authors had detected its presence across the membrane. Yet when mannitol was included as a hydroxyl radical scavenger, killing was suppressed only in the absence of the PTFE membrane. Based on these results, the authors conceived that hydrogen peroxide rather than the hydroxyl radicals plays the major role in the long-range bactericidal effect. Paradoxically, they report that the level of H_2O_2 detected during the experiments was below that usually required to kill bacteria.

Aside from the destruction of cell structure, another possible cause of cell death by illuminated TiO_2 particles could be their detrimental effects on DNA and RNA. Photocatalytic oxidation of a wide range of organic compounds has been observed. Therefore it is therefore not surprising that DNA and RNA can be damaged.[118,120,121] *In vitro* studies using supercoiled plasmid DNA with TiO_2 and near UV illumination by Dunford and coworkers demonstrated that the plasmid DNA was converted first to the relaxed form and later to the linear form, indicating strand breakage.[120] DNA strand breakage is a lethal event. Neither the addition of catalase nor SOD alleviated the strand breakage. When either dimethyl sulfoxide or mannitol were included in the reaction mixture (both which are known to be hydroxyl radical scavengers) the extent of damage to DNA was significantly reduced. Based on these findings the authors were convinced the hydroxyl radical was responsible for the damage and not the more easily diffusible species such as superoxide or hydrogen peroxide. Further analysis of the damaged DNA revealed that the guanine bases of those molecules had been altered.

By irradiating calf thymus DNA containing TiO_2 with UVA light, Wamer and his group detected the hydroxylation of guanine bases.[121] The degree of hydroxylation correlated positively to the amount of TiO_2 used and the intensity of the incident light. Various investigators routinely used the formation of hydroxylation end products of guanine residues from both DNA and RNA as a sensitive probe to determine the extent of oxidative stress.[175] Using human skin fibroblast cells for the *in vivo* studies under the same experimental conditions, Wamer and his coworkers discovered a significant level of hydroxylation of cellular RNA, as had been expected, but no hydroxylation of cellular DNA was detected.[121] Treatment with TiO_2 and UV-A light resulted in 85% cytotoxicity to the human skin fibroblast cells. The authors offered several explanations to the higher oxidative damage to RNA as opposed to DNA: 1) cells possess various repair pathways for DNA molecules, yet no comparable repair mechanisms are known to exist for RNA molecules,[176] and 2) cellular RNA is distributed in the cytoplasm while the DNA in eucaryotic cells is compartmentalized in the nucleus and not readily accessible to either TiO_2 particles, nor to the short-lived ROS. It is important note, as discussed previously, that procaryotic cell structure is not as compartmentalized as are eucaryotic cells. The DNA molecules in procaryotic cells is attached directly onto the cell membrane and is in theory more susceptible to oxidative damage than in the more highly evolved eucaryotic cells.

Hidaka and coworkers provided more direct measurements regarding the fate of DNA and RNA molecules undergoing TiO_2 photochemical damage.[118] By exposing either purine or pyrimidine bases to TiO_2 and light from a 100W Hg lamp, they detected the formation of NO_3^- and NH_4^+ ion species. When native DNA and RNA molecules were subject to the same conditions the authors also detected the transient formation of unidentified peroxide species along with phosphate and carbon dioxide. Release of phosphate suggests the breakdown of the sugar-phosphate backbone of the DNA and RNA molecules, whereas the presence of CO_2 indicates that some mineralization has been realized. Indeed, the authors provided convincing evidence of mineralization reaction as illustrated by the scanning electron micrographs of the DNA strand, clearly demonstrating DNA decomposition within one to three hours following exposure to TiO_2 and light.

In summary, there is conflicting evidence in the literature as to which reactive oxygen species are directly involved in the photo killing process. One school of thought is that hydrogen peroxide maybe more involved than can be accounted for by its oxidative capability, perhaps due to the generation of hydroxyl radicals by means of the Fenton reaction. This may be a possibility since biological systems normally contain Fe^{++}, and cells generally have an abundance of low redox

reducing equivalents. In addition, hydrogen peroxide and superoxide in the presence of catalytic amounts of ferric ion can participate in the iron-catalyzed Haber-Weiss reaction to yield more hydroxyl radicals. More work is needed to clarify which specie or species contributes to the photocatalytic activity.

Regardless of the oxidative species involved, there is substantial evidence that cell membrane damage is the direct result of oxidative damage. The cell membrane contains unsaturated phospholipid and, therefore, is the potential target leading to lipid peroxidation. The detrimental impact of lipid peroxidation to all forms of life has been well documented in the literature.[39] Once the cell membrane barrier is compromised or upon entry into the cell via phagocytosis, TiO_2 particles can exert oxidative actions directly on all the essential components in the cytoplasm.

Patents

The patent literature is rarely included in review articles, however, the background and claims in a patent can be a rich source of ideas and show the direction in innovation and thinking about applications of a technology. The patents in the photocatalytic field in general and those related to cell killing are heavily weighted toward high value applications and those with large potential markets. Methods of immobilizing photocatalysts for applications in both water and air applications are of particular interest. Sterilizing and self-cleaning surfaces are areas of growing activity. Table VI provides titles and references to the world patent literature related to photocatalytic disinfection and hints at the diverse applications that are being considered. Japanese companies and research organizations are responsible for most of the patent activity in this subset of photocatalytic applications as they are in the entire field.

Summary

The work done to date on photocatalytic disinfection using irradiated titanium dioxide has established that the method is effective for killing a wide range of organisms and cell types. The process is applicable to aqueous and air phases and can be used *in vivo*. The broad picture of the mechanism(s) by which cells can be damaged has been revealed. The effects that have been observed are consistent with reactive oxygen species such as those normally associated with

TABLE VI. Patents containing claims or information related to photocatalytic disinfection or cell killing

Patent title	Chemical Abstracts Reference (volume:abstract number)	Reference number
Filters bearing titania-based photocatalytic surface layer for air conditioning	*127:310900*	177
Furnitures containing photocatalyst for deodorization and sterilization	*127:180364*	178
Photocatalytic reactor with flexible supports	*123:264984*	179
Drying apparatus for dishes and control of the apparatus	*127:137486*	180
Heat-resistant antibacterial odor-absorbing agents for finishing fabrics and treating fabrics using them by simplified process	*128:35952*	181
Supporting titanium oxide particles on base materials for formation of photocatalyst layers	*125:66174*	182
Photocatalytic TiO_2-coated material and fabrication thereof	*125:127572*	183
Photocatalysts and method for applying the catalysts	*123:40329*	184
Process for killing cells	--	185
Antimicrobial and weather-resistant coating compositions	*127:36048*	186
Photocatalyst and process for purifying water	*123:40630*	187
Materials for construction of hospitals for preventing infections	*122:170302*	188
Architectural material using metal oxide exhibiting photocatalytic activity	*121:140271*	189
A redox method and membrane-separated photoelectrochemical cell	*109:63262b*	190
Apparatus for purification of bath water with photocatalyst	*124:352185*	191
Photo-catalytic device with titania film for purification of air and water	*126:190684*	192
Metal oxide photocatalysts and their manufacture	*125:127573*	193
Photocatalyst and multifunctional material using it	*114:235835n*	194
Apparatus for purification and disinfection of waters in pool and bath with photocatalyst	*126:135416*	195

(continued)

TABLE VI. Continued

Patent title	Chemical Abstracts Reference (volume:abstract number)	Reference number
Pool water and bath water disinfecting systems	*126:242536*	196
Titanium oxide photocatalyst and method of producing the same	*123:241818*	197
Disinfection apparatus in water purification	*114:88375g*	198
Photocatalytic device	*114:130229q*	199
Air treating method using photocatalyst under interior illumination	*121:140666*	200
Method and reactor for photochemical and photobiological processes using solar energy, useful for drinking water disinfection	*126:242562*	201
Cleaning and regenerating photooxidation catalysts for Water Treatment	*123:208376*	202
Disinfection and deodorization box having titania photocatalyst for air purification	*126:254782*	203
Apparatus for air purification using stacked filters	*128:285892*	204
Apparatus for water treatment, ultraviolet lamp with photocatalytic coating therefor, and its manufacture	*129:32108*	205
Water sterilization using photocatalyst under ultraviolet-light radiation	*129:32107*	206
Manufacture of metal material having photocatalytic activation characteristics	*129:47460*	207
Deodorization disinfection sheets	*129:99205*	208
Photoactive water treatment agents and containers for them	*128:171915*	209
Air-conditioning apparatus equipped with deodorization and anti-bacterial function	*129:112721*	210
Photocatalyst reactive fibers and photoreaction apparatus for deodorization and disinfection	*128:234537*	211

irradiated TiO_2. These may attack from the outside of cells or be generated on the inside of a cell if TiO_2 particles are absorbed by the cell. A universal set of optimum conditions for aqueous phase disinfection does not exist. At present one can only conclude that the treatment conditions are highly dependent on reactor configuration, light source, type of TiO_2, components of the

medium used, and organism. It is important that workers in the field continue to strive for clearly defined conditions for experiments so that comparisons can be made between organisms. The effectiveness of some light sources that are not rated to have output within the band gap of anatase or rutile phases suggests that cell killing might be effective at very low doses of UV light such as might "leak" from an ordinary fluorescent lamp. Thus far it appears that the further one moves away from controlled laboratory media, the more challenging it becomes to kill microorganisms. In the wild, organisms equip themselves with defenses that are more difficult to overcome. Inherent to the photocatalytic method are multiple modes of action which can operate simultaneously--adsorption/trapping, reactive oxygen species, and direct and indirect photochemistry. Control of bioaerosols by a photocatalytic process is only beginning to be studied. The potential for water and air disinfection, self-cleaning surfaces, and dental and medical applications has been established. It remains to be seen if the efficiency and selectivity required by potential applications can be achieved.

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