THE EFFECT OF ANATASE ON RUTILE $\text{TiO}_2$
NANOFLOWERS TOWARDS ITS PHOTO-CATALYTIC ACTIVITY ON CANCER CELLS

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THE EFFECT OF ANATASE ON RUTILE TiO$_2$ NANOFLOWERS TOWARDS ITS PHOTO-CATALYTIC ACTIVITY ON CANCER CELLS

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This thesis was dedicated to my parents, parents in-law, my husband; Mohd Ridzuan Abdullah and my son Ahmad Haziq Mohd Ridzuan
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ABSTRACT

Titanium dioxide (TiO$_2$) rutile nanoflowers were fabricated using acidic hydrothermal method. TiO$_2$ nanoflowers were successfully grown on fluorine doped tin oxide (FTO) substrates. The hydrothermal reaction time and amount of titanium butoxide (TBOT) precursors were optimized to be 10 hours and 2 ml, respectively. Fabrication of TiO$_2$ using spray pyrolysis technique produced TiO$_2$ nanoparticles. The surface morphology of the TiO$_2$ nanoflowers and nanoparticles were studied using Field Emission Scanning Electron Microscope (FESEM) to detect the changes in surface morphology as a result of varied parameters. The crystalline phases of TiO$_2$ samples were investigated using X-ray Diffractions (XRD). From the XRD analysis, TiO$_2$ nanoflowers produced using the hydrothermal method were of rutile phase while TiO$_2$ nanoparticles produced using the spray pyrolysis technique were of anatase phase. Thus, when the two fabrication processes were mixed, anatase-rutile phased TiO$_2$ particles were produced. Photo-degradation of TiO$_2$ nanoflowers were analyzed using methyl orange dye. UV-Vis-NIR spectrophotometer was used to observe the absorbance of methyl orange before and after UV light exposure to the TiO$_2$ samples. The degradation rates of methyl orange by rutile, anatase-rutile and anatase were 4.35%, 88.8% and 98%, respectively. Thereon, cervical cancer cells were exposed to the TiO$_2$ samples to check the biocompatibility of the fabricated TiO$_2$. The cervical cancer cells showed biocompatibility towards the fabricated TiO$_2$. Lastly, photo-catalytic activity of the fabricated TiO$_2$ was studied on cancer cells. The fabricated TiO$_2$ showed little effect to the cancer cells as no change in cell size were observed after UV exposure due to the low electron excitation on TiO$_2$ surface. Thus, the photo-catalysis reaction did not occur.
ABSTRAK

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<tr>
<td>TiO₂</td>
<td>Titanium dioxide</td>
</tr>
<tr>
<td>TBOT</td>
<td>Titanium butoxide</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>CTAB</td>
<td>Cetyl trimethyl-ammonium bromide</td>
</tr>
<tr>
<td>FTO</td>
<td>Fluorine doped tin oxide</td>
</tr>
<tr>
<td>MO</td>
<td>Methyl orange</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet</td>
</tr>
<tr>
<td>NCR</td>
<td>National Cancer Registry</td>
</tr>
<tr>
<td>FESEM</td>
<td>Field emission scanning electron microscope</td>
</tr>
<tr>
<td>XRD</td>
<td>Dispersion X-ray</td>
</tr>
<tr>
<td>DSSC</td>
<td>Dye sensitized solar cells</td>
</tr>
<tr>
<td>VB</td>
<td>Valence band</td>
</tr>
<tr>
<td>CB</td>
<td>Conduction band</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle’s Media</td>
</tr>
<tr>
<td>HBSS</td>
<td>Hanks’s Balance Salt Solution</td>
</tr>
<tr>
<td>FCS</td>
<td>Fetal calf serum</td>
</tr>
<tr>
<td>DAPI</td>
<td>4’, 6-diamidino-2-phenylindo</td>
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Journal / Proceedings:


(d) Noor Sakinah Khalid, Soo Ren How, Mohd Khairul Ahmad. “Effect of Deposition Temperature on Surface Morphology and Electrical Properties of Fluorine Doped Tin Oxide (FTO) Thin Film by Spray Pyrolysis


LIST OF AWARDS

(i) Bronze Medal in Invention & Innovation Awards 2014, Malaysian Technology Expo 2014

(ii) Bronze Medal in Research and Innovation Festival 2013 (R&I Fest UTHM)

(iii) Bronze Medal in Research and Innovation Festival 2014 (R&I Fest UTHM)
CHAPTER 1

INTRODUCTION

1.1 Background of study


The first finding on photo-catalytic reaction on living organism was reported in 1985 by Matsunaga et al., where it was reported that photo-activated TiO$_2$ is effective at killing bacteria [5]. In 1995, Fujishima et al. discovered that TiO$_2$ attains superhydrophilic characteristic after UV irradiation [6]. Following the discovery, in 1997, a hydrophilic surface for self-cleaning application was developed using UV irradiated TiO$_2$ by Wang and his colleagues [7].

Nowadays, there is a high demand for metal oxide semiconductors as photo-catalyst. Titanium dioxide is the most stable metal oxide semiconductor in atmospheric conditions. Extensive research has been conducted on the photocatalytic ability of anatase TiO$_2$. TiO$_2$ photocatalysis finds application in self-cleaning products [8], dye-sensitized solar cells (DSSC) [3-4], anti-microbial products [5-6], water treatment and anti-cancer drugs [7-9]. Photocatalytic ability of TiO$_2$ is influenced by its structure, shape and crystallite phase [10-12]. Anatase TiO$_2$ is the more popular TiO$_2$ phase for photo-catalysis applications. However, the disadvantage
of this phase is that it has an inconvenient shape for removal after a photo-catalysis reaction. In this study, rutile TiO$_2$ was grown on an active substrate which is fluorine doped tin oxide (FTO) substrate. Statically grown rutile TiO$_2$ can easily be removed after photo-catalytic activity. Moreover, anatase TiO$_2$ is metastable and tends to transform into rutile phase at higher temperatures. However, mixing the two phases could have a synergistic effect on its photo-catalytic activity. Thus, the growth of anatase TiO$_2$ on rutile TiO$_2$ nanostructures was studied and optimized to achieve the objectives that will be explained later.

The cancer cells that were used in this study were HeLa cervical cancer cells. HeLa cell line was derived from cervical cancer cells taken from Henrietta Lacks, an African-American woman who died from cervical cancer in 1951. HeLa cervical cancer cells were chosen as the subject for TiO$_2$ photo-catalysis study due to the urgency for cervical cancer treatment. This is in reference to a statistic by the National Cancer Registry (NCR) which reported cervical cancer as the third most common cancers in female in 2007 [19].

1.2 Problem statements

Several studies have been reported on the use of anatase TiO$_2$ structures, namely TiO$_2$ nanotubes and TiO$_2$ nanoparticles, for photo-catalytic killing of cancer cells [7-8, 16]. Cancer cell membranes are composed of phospholipid bilayer, which is a thin outer layer that separates the cell from its environment. During a photo-catalysis process, a lot of free radicals hydroxyl groups (OH·) are produced. A free radical hydroxyl group contains a single unpaired electron which makes it extremely reactive with proteins, lipids and carbohydrates. Free radical hydroxyl groups produced during the photo-catalysis process then attacks the membrane of cancer cells, causing rupture and degradation of cell membrane properties.

The drawback of anatase nanotubes and nanoparticles in previous studies is the difficulty in removing TiO$_2$ particles from the photo-catalytic reaction system and the need for an additional catalyst removal step. Furthermore, anatase TiO$_2$ structure is unstable and may transform to rutile TiO$_2$ at higher temperatures. Rutile TiO$_2$ have a lower band gap energy and a higher electron mobility in comparison to anatase such that more free radical hydroxyl groups are generated with rutile TiO$_2$ as catalyst.
than anatase TiO$_2$ [21]. Higher concentration of free radical hydroxyl groups could enhance photo-catalytic activity. Thus in this study, photo-catalyst comprising a mixture of anatase and rutile TiO$_2$ was proposed as it could possibly increase photo-catalytic activity towards the cancer cells.

TiO$_2$ nanoflowers have a larger surface area in comparison to TiO$_2$ nanoparticles. This is because the petals of the nanoflowers have a larger surface area to volume ratio in comparison to spherical TiO$_2$ nanoparticles. The TiO$_2$ structure with a larger surface area will generate more free radical hydroxyl groups. Thus, the growth of TiO$_2$ in nanoflower structure is favorable as it would increase photo-catalytic activity of TiO$_2$ towards cancer cells.

1.3 Objectives of the study

Below are the objectives of this research:

(i) To fabricate TiO$_2$ anatase nanoparticles on top of rutile nanoflowers using hydrothermal and spray pyrolysis method.
(ii) To study the effect of photo-degradation of methyl orange (MO) using TiO$_2$ nanostructure.
(iii) To investigate the biocompatibility of HeLa cells grown on TiO$_2$ nanostructures.
(iv) To apply photo-catalytic activity on HeLa cells using TiO$_2$ nanostructures after UV radiation.

1.4 Research scope

Hydrothermal method was chosen as the fabrication method for rutile TiO$_2$ because it is simple and may be performed in a closed system. Spray pyrolysis method was chosen as the fabrication method for anatase TiO$_2$ as spray method is a simple and easy method to obtain anatase TiO$_2$ nanoparticles where it involves spraying a precursor solution on rutile TiO$_2$ substrate.

TiO$_2$ nanostructures were characterized using Field Emission Scanning Electron Microscope (FESEM) to observe the morphology of TiO$_2$ on its surface and at its cross-section. It was to determine the size of TiO$_2$ nanostructures that had been
fabricated. X-ray Dispersion (XRD) was used to confirm the crystallite phase of the TiO$_2$ nanostructures after fabrication.

Moreover, to study the photo-degradation property of TiO$_2$ on MO ultra violet visible near infra-red (UV-Vis-NIR) spectrophotometer was used. Additionally, a study on the biocompatibility of TiO$_2$ nanostructures was performed by adding the TiO$_2$ nanostructures to HeLa cells. Furthermore, photo-catalytic effect of the TiO$_2$ nanostructures on HeLa cells was studied through UV light excitation on HeLa cells exposed to photo-excited TiO$_2$ nanostructures.

1.5 Research contribution

This study proposed the growth of anatase on rutile TiO$_2$ nanostructure to enhance photo-catalytic activity. It has been discovered that the rutile TiO$_2$ nanoflowers grown on FTO substrate provided the base for anatase growth as the rutile TiO$_2$ nanoflowers consist of active sites. After a photo-catalytic reaction has finished, the TiO$_2$ photo-catalyst may be easily removed from the reaction chamber. This application could use cervix as a target area.

As mentioned in section 1.2, rutile TiO$_2$ has a higher electron mobility than anatase. This property will help to accelerate electrons movements to the anatase TiO$_2$ grown on top of it and increase electron excitation at the contact surface between cells and TiO$_2$. 
CHAPTER 2

LITERATURE REVIEW

In this chapter, titanium dioxide nanostructures and various methods used to prepare TiO$_2$ nanostructured thin film were discussed. The photo-catalytic process that involves TiO$_2$ was described. Then, this chapter also discussed about biocompatibility and photo-toxicity of TiO$_2$ towards living cells. Moreover, the working principles of FESEM, XRD, UV-VIS NIR and fluorescence microscope were explained. Then, what were cancer cells about were reviewed. Studies on the effect of TiO$_2$ on cancer cells by previous research works were reviewed for reference purposes. Lastly, the possible applications of TiO$_2$ were described.

2.1 Titanium dioxide nanostructures

The performance of titanium dioxide or titania as metal oxide semiconductor has been widely studied due to its special photo-catalytic properties [20-22]. TiO$_2$ has three different forms of crystallite structure, namely, anatase, rutile and brookite [3-4, 23]. Anatase and brookite are metastable and can be transformed to rutile phase when annealed at high temperatures [21, 24-26]. However, the fabrication of pure brookite without the presence of anatase or rutile phases is difficult to achieve, thus brookite is rarely studied. Rutile, on the other hand, is the most stable form of TiO$_2$. Conventionally, temperatures of around 700 °C and above are used to produce pure rutile TiO$_2$ structure [23, 25].

It has been reported that the transition onset temperature needs to be around 600 °C to transform anatase to rutile as shown in Figure 2.1 [22].
Figure 2.1: Reaction boundaries of phase transition in TiO$_2$ [22].

The crystal structure of anatase consists of 4 edges sharing while rutile consists of 2 edges sharing, as shown in Figure 2.2. This is as anatase and rutile have 4 and 2 atoms per unit cell, respectively [22]. Both anatase and rutile structures are tetragonal. The band gap energy for TiO$_2$ is large, around 3.0 eV, depending on its form. The specific values are 3.26 and 3.05 eV for anatase and rutile, respectively [21, 25, 27]. The difference is attributed to the arrangement order of its structure, as shown in Figure 2.2 [22].

Figure 2.2: Crystal structures of (a) anatase, (b) rutile and (c) brookite [22].

As shown in Table 2.1, TiO$_2$ has a high refractive index which results in high reflectivity from its surface. This property allows TiO$_2$ to absorb more light, thus, making it an effective photo-catalyst.
Table 2.1: Bulk properties of TiO$_2$ crystal phases [28].

<table>
<thead>
<tr>
<th>Crystal structure</th>
<th>System</th>
<th>Band gap (eV)</th>
<th>Density (kg/m$^3$)</th>
<th>Refractive index</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$n_g$ $n_p$</td>
</tr>
<tr>
<td>Rutile</td>
<td>Tetragonal</td>
<td>3.05</td>
<td>4240</td>
<td>2.946 2.650</td>
</tr>
<tr>
<td>Anatase</td>
<td>Tetragonal</td>
<td>3.26</td>
<td>3830</td>
<td>2.568 2.658</td>
</tr>
<tr>
<td>Brookite</td>
<td>Rhombohedral</td>
<td>-</td>
<td>4170</td>
<td>2.809 2.677</td>
</tr>
</tbody>
</table>

A lot of research works have been done on the nanostructures of TiO$_2$ in a variety of area. TiO$_2$ nanostructures include nanoparticles, nanorods, nanobelts, nanowires and nanoflowers [3, 25, 28-30]. TiO$_2$ nanoparticles are usually made by sol-gel method and spray pyrolysis deposition technique [31-32]. Normally, hydrothermal method is utilized to produce nanorods, nanowires, nanobelts and nanoflowers due to the ability to control pressure and temperature of the reaction in order to control the shape and size of the resultant TiO$_2$.

2.2 Different techniques for preparation of nanostructured TiO$_2$ thin film

In this section, the preparation of nanostructured TiO$_2$ thin film is discussed. There are a number of methods available for fabricating nanostructures and particles such as sol-gel method assisted with spin coating, spray pyrolysis deposition technique, DC or magnetron sputtering and hydrothermal method.

2.2.1 Sol-gel method

Sol-gel process is defined as the process for transiting liquid “sol” into “gel” phase. A sol is formed after a series of hydrolysis and condensation processes of precursors. After that, the sol particles are condensed to form a gel. The gel is applied to heat treatment and drying, until it transforms into dense particles. Sol-gel process generally uses hydrolysis, condensation and solvent reactions [35]. Puangrat et al. reported that sol-gel preparation produces nanostructured TiO$_2$ of anatase phase only and the thin film was coarse [36]. Moreover, in a paper reported by Venkatachalam et al., it was reported that they were able to fabricate nanostructured anatase TiO$_2$ after calcination at 300 °C. The obtained nanostructured anatase TiO$_2$ transformed to rutile phase when annealed at 800 °C [37].
2.2.2 Spray pyrolysis deposition technique

In spray deposition process, the solution is atomized by at appropriate pressure so that it deposits are formed on the substrates in tiny droplets. Careful selection of the solution is needed so that only undesired product will be evaporated at the temperature of deposition [42-43]. The characteristics of the produced thin film depend on spray rate, deposition temperature, droplet size and distance of spray nozzle to substrates [39]. Figure 2.3 shows a schematic diagram of a typical spray deposition setup [38].

![Schematic diagram of spray deposition setup](image)

Figure 2.3: Schematic diagram of spray deposition setup [38].

Ranga et al. reported TiO$_2$ nanoparticles fabricated by spray pyrolysis method. They produced TiO$_2$ nanostructures that has high porosity and with anatase crystallite structure [40]. Besides, there was study done on modeling spray pyrolysis for gas sensors. It means that spray deposition is better for sensing application.

2.2.3 Sputtering

The mechanism of sputtering involves bombarding plasma onto a certain target on the substrates. Ching-Hua et al. published a paper on the principle of sputter systems. Cations from argon (Ar$^+$) are discharged by plasma at high temperatures. This is followed by control using an electric field to bombard the target. The particles from the target metal were then deposited on the substrate surface. To produce TiO$_2$ thin
film, pure TiO$_2$ target (99.99 \%) was used [18]. Moreover, Kaczmarek et al. also reported their work on the fabrication of TiO$_2$ nanoparticles using modified magnetron sputtering. Their work yielded anatase and rutile by using hot target and high energy, respectively [41]. Another finding by Valencia et al. reported the use of RF magnetron sputtering to produce anatase and rutile by inductively coupled radio frequency plasma of calibrated argon/oxygen mixtures without post-annealing [42]. Figure 2.4 shows the plasma reactor used in a sputtering system.

![Figure 2.4: Diagram of plasma reactor [42].](image)

### 2.2.4 Hydrothermal method

The definition of hydrothermal is a process in which a solution is reacted in a closed system under controlled temperature and pressure with water as the solvent [43]. The internal pressure is usually determined by the amount of solution and temperature. By using hydrothermal, many structures could be formed such as nanowires, nanotubes, nanorods, needle-like structures, nanoflowers, and snowflake-like structures [3, 28-30, 46-48]. Moreover, hydrothermal method is the best method to prepare TiO$_2$ nanostructures on conductive substrates due to the presence of active sites on the substrate’s surface. Thus, nucleation of crystallite TiO$_2$ nanostuctures may take place [28-30].

There are a few parameters that could influence the properties of TiO$_2$ such as structure, crystallite phase, pH of solution, and size of particles. TiO$_2$ anatase are
produced when the solution is a weak acid or an alkaline solvent [34, 39]. However when the solution is highly acidic rutile TiO₂ particles are produced, as reported in previous studies [3, 11, 20, 25, 49].

The general chemical reaction occurring in the hydrolysis reaction of Ti precursor is shown below:

\[
Ti(OR)_4 + H_2O \rightarrow TiO_2 + ROH
\]  

(2.1)

After hydrolysis process has occurred, the pressure and temperature in the hydrothermal reaction chamber influences the shape of the TiO₂ precipitated onto the substrate to be crystallite nanostructures. A report by Wu et al. discusses the formation process of anatase TiO₂ on FTO glass. The formation starts with nucleation of TiO₂ on the active sites. They then grew into nanorods with time [31]. Whereas, a study conducted by Hong et al. discussed the fabrication of rutile TiO₂ nanorods [32]. Figure 2.5 shows the schematic diagram based on reports by Wu et al. and Hong et al..

Figure 2.5: Schematic diagram of TiO₂ nanostructures using hydrothermal method by Wu (left) and Hong (right) [29, 30].

As stated in the previous paragraph, acid solution and alkaline solution produces rutile and anatase TiO₂, respectively. A study by Zhiqiao et al. found that hydrothermal synthesis using strong hydrochloric acid (HCl) produced rutile nanorods due to the high number of hydroxyl group (OH) ligands thus producing two edge-shared bonding rutile structure [17]. Whereas, Chin et al. used alkaline sodium hydroxide (NaOH) and obtained anatase nanotubes where the NaOH breaks the Ti-O-Ti bond to form four sharing edge bond of TiO₂ [49]. So, for this study, the author had used HCl as a chelating agent using hydrothermal method to produce rutile TiO₂ nanostructures.
2.3 Photo-catalytic properties of TiO$_2$

The photo excited state of a semiconductor is generally unstable and can be easily broken down. However, TiO$_2$ is stable when it is photo-excited. Thus, TiO$_2$ can be a good photo-catalyst [50]. In the previous studies, there were a few reported applications of TiO$_2$ as a photo-catalyst. It was used in water treatment in which it could kill the microorganisms and bacteria in contaminated water [5, 36, 51-52], water splitting to produce hydrogen gas [11, 27], and for cancer cell treatments [7-8, 16]. These are interesting areas to be explored.

Semiconductor with photo-catalytic unique property is characterized by its valence band (VB) and conductor band (CB). The area between VB and CB is called a band gap where there are no electrons in that state. However, electrons can jump from VB to CB when the semiconductor absorbs photons with energy greater than or equal to its band gap energy, $E_g$ [53]. As shown in Figure 2.6, the photo-catalytic reaction of TiO$_2$ starts upon exposure to light. The exposure generated hole-electron pairs. The electrons at VB are excited to CB when the light energy is the same or greater than the band gap energy. Thus, the processes of oxidation and reduction occurs when the photo-generated electron reaches the reaction sites [53, 54]. The electrons in the VB of TiO$_2$ could be excited with light having a wavelength in the range of UV light ($\lambda<400$ nm) which corresponds to TiO$_2$’s band gap i.e. 3.2 and 3.0 eV for rutile and anatase, respectively [27, 51, 55-57].

![Figure 2.6: Schematic diagram of TiO$_2$ photo-catalyst (reproduced figure).](image)
The mechanisms of the photo-catalytic reaction may be simplified in the chemical reactions below [7, 8]. When UV light excites TiO$_2$ particle, it produces hole$^+$ at the VB and excites one electron to the CB as in equation (2.2) to (2.5).

$$TiO_2 + hv \text{ (UV light)} \rightarrow h^+ + \bar{e}$$  \hspace{1cm} (2.2)

$$TiO_2^- + O_2 + H^+ \rightarrow TiO_2 + HO_2^-$$  \hspace{1cm} (2.3)

$$TiO_2^- + H_2O_2 + H^+ \rightarrow TiO_2 + H_2O + OH^*$$  \hspace{1cm} (2.4)

$$\bar{e} + O_2 \rightarrow O_2^- + H_2O \rightarrow OH^*$$  \hspace{1cm} (2.5)

At the CB, the hole reacts with oxygen and hydrogen ions to produce free radical hydroxyl group. While at the VB, the electron reacts with oxygen and produces superoxide anions which later reacts with water molecules to produce free radicals hydroxyl groups. The free radical hydroxyl groups produced are reactive to organic substances [7-8, 16, 58].

As reported in previous studies, micro-organisms in waste water consist of organic substances as well as single cells. What makes them organic is that the membrane of the cells is made from phospholipid bilayer. When generated free radical hydroxyl groups comes into close contact with the microorganisms, the cell wall is the first site attacked [57]. Thereon, the free radical hydroxyl groups will eventually kill microorganisms, bacteria and cancer cells. Those organisms have a phospholipid bilayer made from organic compounds. The membrane of cancer cells may also be attacked by free radical hydroxyl groups released from photo-catalyzed TiO$_2$ particles. The photo-catalytic mechanism is illustrated using a schematic diagram shown in Figure 2.6.

Basically, UV light consist of three main regions which are UVA, UVB and UVC with specific wavelengths range of 315 to 400 nm, 280 to 314 nm and 279 to 100 nm, respectively. UVA is not harmful to our body but too much exposure can cause aging of skin and eventually can cause cell mutation. On the other hand, exposure to UVB in long term can cause burning and skin reddening which can stimulate melanocytes cells to produce pigment in skin cells [59]. However UVC is very harmful to the cells as it can kill the cells by direct exposure. UVC is widely used to kill germicides and to sterilize safety cabinets. Generally, in our atmosphere UVB and UVC are filtered by the ozone layer and only UVA may reach the ground. However, UVA is less harmful to our skin since we cover ourselves with clothes [67, 68].
In this study, UVA was used to activate photo-catalytic activity of TiO$_2$ nanostructures against cells. The energy from UVA is enough to activate the electrons at the VB of the TiO$_2$ in order for the electrons to be excited to CB for photo-catalysis to occur since the band gap energy of TiO$_2$ was in range of 3.0 to 3.2 eV and it only absorbs UV light [52]. Methyl orange was used as an indicator to observe the changes to the main absorption peak which is at 465 nm [11, 50].

2.4 Biocompatibility of TiO$_2$

Biocompatibility is one of the important issue that needs to be addressed when applying inorganic materials into the human body. The material needs to be non-toxic, non-carcinogenic, non-thrombogenic, non-immunogenic, non-irritant and so on [61]. Several research work has been reported on the biocompatibility of TiO$_2$. Francisco et al. stated that TiO$_2$ thin film was appropriate to culture neuron cells [62]. Furthermore, a study reported that TiO$_2$ nanoparticles have a stable hydrophilic biocompatibility to human dermal fibroblast cells [63]. In addition, S. Sangeetha et al. reported that TiO$_2$ are biocompatible to human blood and may be used in implants [64]. Moreover, Hangzhou et al. reported antibacterial ability and biocompatibility of TiO$_2$ nanotubes as implants [65]. Thus, we can conclude that TiO$_2$ is biocompatible to living cells due to its inert property.

2.5 Photo-toxicity of TiO$_2$

In terms of photo-toxicity, TiO$_2$ could be toxic due to the production of reactive oxygen species (ROS) by the photo-catalytic process mentioned above. This is as ROS are very reactive to organic substances. Jun-Jie et al. found TiO$_2$ to be photo-toxic to human skin keratinocytes when irradiated with UVA at 5.0 J/cm$^2$ [66]. Additionally, Alexandra et al. reported that TiO$_2$ became toxic to cells due to the presence of ROS upon irradiation of UVA [67]. It can be concluded that TiO$_2$ is not harmful to living cells unless irradiated with UV light at which they will go through photo-catalytic process on the surface of cell wall membrane. Therefore, in this study TiO$_2$ is to be applied to cancer cells and the selected area will be exposed to UV light in order to generate ROS and kill the cells.
2.6 Working principles of devices used for characterization

In this section, the theory and concept of the devices used for characterization such as FESEM, XRD, UV-VIS NIR Spectrophotometer and fluorescence microscope is discussed for better understanding on how these devices work to obtain the data needed.

2.6.1 Field emission scanning electron microscope (FESEM)

The principal of electron microscope is almost the same as conventional microscope but differs in its energy source. Electron microscope uses electrons as an energy source while basic microscope utilizes visible light as the energy source. Visible light is limited to its wavelength range while accelerated electrons have a very short wavelength. Thus, it is possible for electron microscopes to observe morphology in micro or nano-scale [68].

FESEM is a type of electron microscope capable for analyzing a surface by scanning it with high energy beams of electrons in parallel scan patterns. It requires high vacuum condition so that the electrons could be emitted directly to the sample. Firstly, electrons are emitted from a tungsten filament. Then they are made to exit the electron gun where they are then focused and confined into a thin focus. The monochromatic beam uses metal aperture and magnetic lenses. Lastly, a detector detects the electrons from the sample and collects the signals to produce an image of sample [72-73]. The surface morphology of TiO$_2$ nanostructures that produced in this study were characterized using FESEM JEOL JSM-7600F.

2.6.2 X-ray diffraction (XRD)

The mechanism of XRD applies electromagnetic radiation (x-ray) which has smaller a wavelength and a higher energy in comparison to visible light. When the x-ray beam hits an atom, the electrons around the atom start to vibrate with the same incidence as the incoming beam. This result in a destructive interference and hence the combined waves were out of phase. No residual energy is left out of the solid sample. But, the atoms in a crystal are arranged in a regular pattern and there are
some small constructive interference. The remaining waves were in phase. Thus, a
diffracted beam could be described as a beam composed of a large number of
scattered rays commonly reinforcing one another [74-75].

![Figure 2.7: Schematic diagram of Bragg's Law [71].]

The principles of XRD is defined from Bragg’s Law where the beam
reflected from the lower surface travels farther than the one reflected in upper
surface. When the path difference is equal to some integral multiple of the
wavelength, constructive interference occurs. The condition for a constructive
interference is given by Bragg’s law in equation 2.6. A schematic diagram of Bragg’s
Law is shown in Figure 2.7. The angle between the incident beam and the lattice
planes is called θ. The wavelength of the beam is defined by the symbol λ while n is
the order of the lattice. On the other hand, d value is defined as the spacing between
lattice [71].

\[
d = \frac{n\lambda}{2\sin\theta}
\]  

(2.6)

Thus, XRD can be used to determin crystal structures and the intensity of
crystallinity for the TiO₂ nanoflowers sample.

2.6.3 UV-Vis NIR Spectrophotometer

This device was used to measure the absorbance or reflection of a sample. This
spectrometer is capable of measuring wavelength in the ultra violet (300 – 400 nm),
visible (400 – 765 nm) and near-infra red (765 – 3200 nm) regions. The energy
absorbed or emitted by a photon while transitioning from one energy level to another
is provided by the equation 2.7 where $h$ is the Plank’s constant and $v$ is the frequency of the photon.

\[ e = hv \quad (2.7) \]

\[ c = v\lambda \quad (2.8) \]

Thus, \[ E = \frac{hc}{\lambda} \quad (2.9) \]

In essence, the shorter the wavelength, the greater the energy of the photon and vice versa. When white light is shine on a substance, the light may be totally reflected, making the substance appear white or the light may be absorbed, making the substance appear black. Thus, it gives the relationship between light absorption and colour. For methyl orange dye, the range of absorbed wavelength is at 480 nm to 490 nm [72]. As degradation might occur in methyl orange substance, the intensity of the absorption may be reduced as a result of the photo-catalytic activity of TiO$_2$ samples.

### 2.6.4 Fluorescence microscope

Unlike conventional microscopes which uses visible light, this microscope uses florescence as the reflection source. Sample under observation is illuminated with a light of certain wavelength which are then absorbed by fluorophores, causing them to emit light of longer wavelength [73].

The sample is dyed so that it may absorb and emit certain light. The light source used is either mercury lamp or xenon lamp. For example in living cells, the sample are immersed with 4’,6-diamidino-2-phenylindo (DAPI) staining that can only be absorb by the nucleus of cells. Then, when the sample is emitted with a specific light, the nucleus emits blue color, an indication that the cell is alive.

The concept of this microscope is illustrated in the Figure 2.8. It shows the components from the light source to where images are captured.
Cancer is an abnormal growth of cells occurring when the cells split aggressively. According to the NCR, ten leading cancers among the population of Malaysia in 2007 were breast, colorectal, lung, nasopharynx, cervix, lymphoma, leukemia, ovary, stomach and liver cancer [19]. Cervical cancer was the third most common cause of cancer death, after colorectal and breast cancer and was the most common cancer in women, accounting for 8.4% of death in women in 2007 [19].

One of the characteristic of cancer cells is the aggressiveness in proliferation of cells. It means the cells divide themselves vigorously as shown in figure 2.9. When abnormal cells spread at a certain area, they become cancerous, slowly destroying normal cells. Moreover, cancer cells have stronger resistance in comparison to normal cells. Cancer treatment usually involves high doses of drugs and chemical substances in order to overcome the cancer cells [74].
There are many types of cancer cells and it depends on where it develops. For instant, lung cancer occurs in the lungs and colon cancer occurs in the colon. Cervical cancer originates from cancerous cells lining the cervix. The normal cervix cells first develop pre-cancerous changes that will eventually turn them into cancerous cells. Then, the cells start to develop aggressively in the cervix [75]. Cervical cancer cell line, known as HeLa cells, were taken from an African American woman called Henrietta Lacks at 1951. After she died her cervical cancer cells were sub-cultured for research purposed [76].

In this study, the HeLa cells were chosen due to their availability at the market and their high resistance in comparison to normal cells. For development into a medical device, TiO$_2$ nanostructures may be inserted at the end of a fiber optic where the fiber optic may be used in a probe to kill cancer cells in cervix area by using photo-catalytic effect.
2.8 Application of titanium dioxide

In daily uses, TiO$_2$ finds application in paints and sunscreens as white pigments [33-34]. TiO$_2$ particles are used to increase scattering of visible light. TiO$_2$ applications have expanded to important technological areas such as dye-synthesized solar cells (DSSC) [3, 35], environmental areas such as water treatment [5, 36], and medical areas such as self-sterilizing coatings [79]. Furthermore, TiO$_2$ particles are also used in gas sensors, corrosion protective coatings, optical coatings and transistors [80]. Moreover, Chutima et al. reported possible TiO$_2$ nanofibers application in a new area, for anti-microbial and self-cleaning [8]. For gas sensor applications, researchers found that TiO$_2$ has defined structure suitable for highly sensitive and optoelectronic sensors [24]. The most promising application of TiO$_2$ reviewed by Markowska et al. showed the potential of TiO$_2$ to be used in antibacterial, antifungal, antiviral and anticancer fields [47]. This study is aimed at improving the functionality of TiO$_2$ in cancer cell treatment.
CHAPTER 3

METHODOLOGY

Chapter 3 describes and justifies the fabrication methods used for TiO$_2$ nanoflowers and TiO$_2$ nanoparticles, namely, hydrothermal and spray pyrolysis method, and the physical characterization methods used, namely, FESEM and XRD analysis. Next, the photo-catalytic activities of fabricated TiO$_2$ samples were determined using MO under 20 W UV light exposure. This was followed by photo-catalytic study of HeLa cells grown on TiO$_2$ samples.

3.1 Flow chart

The overall experimental work was summarized in a flow chart shown in Figure 3.1. The experimental work was conducted in three main stages, namely, the fabrication stage, the characterization stage and the verification stage.
3.2 Variation of experimental parameters

The parameters used in TiO$_2$ nanoflowers fabrication were hydrothermal reaction time and the amount of titanium butoxide (TBOT). The hydrothermal reaction time were varied from 2 to 24 hours while the amount of TBOT were varied from 1 to 3 ml. The parameters were chosen based from the findings of previous studies. For the photo-catalytic activity study using MO, the parameter used was different TiO$_2$ nanostructures obtained from the varied fabrication methods. For the photo-catalytic study of all the different TiO$_2$ nanostructures on HeLa cells, the UV light exposure time to the cells was varied from 10 to 60 minutes. The parameters are summarized in Table 3.1.
Table 3.1: Variation of experimental parameters

<table>
<thead>
<tr>
<th></th>
<th>Fabrication TiO$_2$ nanostructures</th>
<th>Amount of TBOT (ml)</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hydrothermal time (hours)</td>
<td>2</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>2</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Photo-catalytic MO Different TiO$_2$ samples</td>
<td>TiO$_2$ rutile</td>
<td>TiO$_2$ rutile-anatase</td>
<td>TiO$_2$ anatase</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Photo-catalytic on HeLa Different TiO$_2$ samples</td>
<td>UV time exposure (minutes)</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
</tbody>
</table>

3.3 Fabrication of TiO$_2$ nanoflowers by hydrothermal method

The chemicals used to fabricate TiO$_2$ nanoflowers were used as purchased. Hydrochloric acid (HCl) was used as a chelating agent. TBOT was used as a precursor material for TiO$_2$ nanoflowers while deionized water was used in the hydrolysis reaction. Figure 3.2 (a,b,c) shows the chemicals used in the hydrothermal method. The FTO glass substrates used as templates for growing TiO$_2$ nanoflowers are shown in Figure 3.2 (d). The FTO glass substrates have a resistivity of about 7 Ω/sq. The substrates were cut into 1 cm by width and 1.5 cm by length. A mixture of deionized water, acetone and ethanol at a ratio of 1:1:1 was used to clean the substrates. The substrates were submerged in the mixture and exposed to ultrasonication in an ultrasonic bath for 10 minutes. Thereon, the substrates were dried in ambient air. The volume of HCl, TBOT and deionized water were varied to optimize the structure of the TiO$_2$ nanoflowers.

Firstly, 80 ml of HCl was mixed with 80 ml of deionized water on a hot plate stirrer at 35°C and a stirring speed of 350 rpm. Then 1 ml of TBOT was dropped wisely until the solution became transparent. The cleaned FTO substrates were placed inside Teflon-lined stainless steel autoclave, shown in Figure 3.2 (e). This process is known the hydrothermal method, a process where a precursor solution is subjected to heat treatment in a tight chamber. Then, the autoclave was placed inside
a convection oven and heated at 150 °C for 2, 5, 10 and 24 hours to observe TiO$_2$ structural changes with increasing hydrothermal reaction time.

After the hydrothermal reaction has ended, the autoclave was cooled down to room temperature. The substrates were taken out and rinsed with deionized water. Then, the rinsed substrates were dried in an oven at 100 °C for 20 minutes. The experiment was repeated using 2 and 3 ml of TBOT to monitor changes to the TiO$_2$ structure with an increased amount of TBOT precursor.

![Image](image.png)

Figure 3.2: (a) Hydrochloric acid, (b) deionized water, (c) titanium butoxide as precursor of TiO$_2$ nanoflowers, (d) FTO glass substrate used as growth template, and (e) Teflon lined stainless steel autoclave where hydrothermal reaction takes place

### 3.3.1.1 Characterization of surface morphology and structural phase of TiO$_2$ nanoflowers

The TiO$_2$ samples obtained from hydrothermal method went through surface morphological analysis using FESEM JEOL JSM-7600F. The crystallinity of the sample was tested using XRD PANalytical X-Pert Powder with Cu $\beta$ K$_\alpha$ radiation at 40 kV. The characterization machines used in the experiment are shown in Figure 3.3.
An important thing to remember when using FESEM is that samples have to be in dry state. This is because the condition inside the sample chamber has to be in vacuum to allow the electrons to bombard the sample without any interruptions and generate clear images. Firstly, the sample was placed onto a sample stage and clamped. Then, the air inside the sample chamber was removed until the chamber was fully in vacuum. After that, electrons were bombarded onto the sample and the current was adjusted to obtain clear images.

For characterization using XRD, the sample was placed on a stage holder. The beam angle was adjusted to 5°. Then, x-ray was excited onto the sample. Peaks corresponding to different elements were displayed in a graph showing X-ray intensity plotted against 2θ. It can be interpreted that the higher the intensity, the higher the concentration of that element is present in the sample.

3.4 Fabrication of TiO$_2$ nanoparticles by spray pyrolysis method on top of TiO$_2$ nanoflowers

TiO$_2$ solution was prepared by mixing TiO$_2$ P25 powder with acetic acid in a mortar. The mixture was grinded until thoroughly combined. Then, TKC-303 was added into the mortar and the solution was mixed well before it was transferred into a lightproof bottle. Ethanol and Triton X-100 were added into the solution. The mixture was then ultrasonicated for 30 minutes. TiO$_2$ nanoflowers grown on FTO substrates fabricated in the previous experimental step (fabricated using 2 ml of TBOT and 10 hours hydrothermal reaction time) were used and were aligned on a hotplate with the
REFERENCES


