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Biocompatibility of TiO_2 Nanorods and Nanoparticles on HeLa Cells

(Biokeserasian TiO_2 Nanorod dan Nanopartikel ke atas Sel HeLa)

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ABSTRACT

Titanium dioxide (TiO_2) nanorods and nanoparticles had been successfully done by hydrothermal method and spray pyrolysis deposition technique, respectively. Form XRD results, crystallite structure for TiO_2 nanorods is rutile phase at 2θ degree 27.5° which corresponded to [110] orientation. Whereas, TiO_2 nanoparticles produced anatase phase at 2θ degree 25.3° which corresponded to [110] plane. The structure of nanorods and nanoparticles were characterized using FESEM. The size of nanorods was in the range of 80 to 100 nm. While, the nanoparticles size was ranging from 25 to 35 nm. The HeLa cells were grown on those TiO_2 and were observed under fluorescence microscope. The cells showed healthy sign of growth on TiO_2 nanorods and nanoparticles substrates. Thus, TiO_2 nanorods and nanoparticles are biocompatible to HeLa cells.

Keywords: Biocompatibility; nanoparticles; nanorods; TiO_2

ABSTRACT

Titanium dioksida (TiO_2) berbentuk nanorod dan nanopartikel telah berjaya dihasilkan melalui kaedah hidroterma dan teknik semburan pirolisis. Analisis daripada XRD menunjukkan komposisi untuk TiO_2 nanorod adalah fasa rutil pada 2θ *darjah dengan bacaan 27.5° yang sepadan dengan jujukan [110]. Manakala nanopartikel TiO_2 adalah fasa anatas pada* 2θ *darjah dengan bacaan 25.3°* yang *sepadan dengan jujukan [101]. Bentuk nano-silinder dan nano-partikel telah dianalisis menggunakan FESEM. Saiz nanorod adalah antara 80 dan 100 nm diameter. Selain itu, saiz nano-partikel adalah 25 hingga 35 nm diameter. Kemudian, sel HeLa telah dibiakkan di atas sampel TiO_2 tersebut. Setelah itu, sel HeLa diperhatikan di bawah mikroskop pendarfluor. Keputusan menunjukkan pertumbuhan sel HeLa yang sihat pada TiO_2 nano-silinder dan nano-partikel. Oleh itu, TiO_2 nanorod dan nano-partikel menunjukkan keserasian biologi kepada sel HeLa.*

Kata kunci: Keserasian biologi; nano-partikel; nano-silinder; TiO_2

INTRODUCTION

Titanium dioxide (TiO_2) is a metal oxide semiconductor that has a major role in photo-catalytic process. In a previous research, it was proven useful as a catalyst in photodegradation of organic compound such as for waste water and anti-bacterial application (Seo et al. 2007). TiO_2 has three main structures which were anatase, rutile and brookite (He et al. 2013; Mohd Khairul Ahmad 2012). Rutile is a more stable crystallite compared to anatase and brookite. Anatase is metastable which means it can transform to rutile at certain temperature. The band gap energy for anatase and rutile are 3.0 and 3.2 eV, respectively, (Valencia et al. 2009) which were wide bandgap. However, TiO_2 is chemically inert and was widely used in biomaterial studies such as in dental implant and bone implant. Thus this study was to support the biocompatibility of TiO_2 nanostructures i.e. nanorod and nanoparticles on cells.

The HeLa cells used in this study were originated from a black woman called Henrietta Lacks who died from cancer cervix in 1951. HeLa cell is an immortal cell line that is used in research studies. This cell is easily grown and spread in culture flask due to its cancer properties that make it easily to proliferate (Zielinski 2010). Thus, this cell was chosen to be applied on TiO_2 nanostructures to check the biocompatibility of cells on TiO_2 nanostructures.

In this paper, the TiO_2 samples were prepared by hydrothermal and spray pyrolysis deposition methods to fabricate nanorods and nanoparticles. TiO_2 fabricated by hydrothermal method in acidic solution will give rutile phased TiO_2 nanostructures while, spray pyrolysis method produced TiO_2 anatase. Usually, to prepare TiO_2 rutile phased, it needs high temperature in high pressure environment. But, when the hydrothermal method was introduced, it only used low temperature at atmospheric pressure to get the TiO_2 rutile phase since it take place in closed system in stainless steel autoclave lined with Teflon (Khalid et al. 2015). Spray pyrolysis method will produce nanoparticles that uniformly spread on the substrates. This method has an advantages in producing lots of samples at one time and it can be simply setup in laboratory.

Then, HeLa cells were grown on those TiO_2 nanostructures in order to investigate the biocompatibility of HeLa cells on TiO_2 nanorods and nanoparticles. The results will be discussed later.

Methods

TiO_2 nanorods were fabricated using hydrothermal method while TiO_2 nanoparticles were fabricated by spray pyrolysis deposition technique. The substrates used in this study were FTO glass from Aldrich with ~7Ω/sq resistivity. First, the substrates were cleaned with acetone, ethanol and deionized water of ratio 1:1:1. For TiO_2 nanorods, hydrochloric acid (36.5~38.0% JT Baker), titanium (IV) butoxide (97% Sigma-Aldrich) and deionized water were used as purchased. The mixture was stirred in a beaker for 10 min. After that, the solution was put into teflon-lined stainless steel autoclave in the oven at 150°C for 10 h. The sample was allowed to cool down before the substrates with TiO_2 were rinsed using deionized water. The sample was dried it in oven at 100°C for 1 h.

TiO_2 nanoparticles were done using spray pyrolysis deposition technique. Acetic acid from Sigma-Aldrich, commercialize TiO_2 P25 (25% rutile, 75% anatase), TKC-303 from Tayca corporation, ethanol and triton-X 100 were used. Acetic acid and TiO_2 P25 were mixed in a mortar before TKC-303 was added. After it mixed well, the solution was put into a bottle. Ethanol and triton-X 100 were mixed together with the solution in the bottle and the solution was put for an ultrasonic for 30 min. The solution was sprayed onto the FTO substrates lined on a hot plate set at 150°C. Then, the substrates with TiO_2 nanoparticles were annealed at 600°C for 3 h.

HeLa cells (ATTC: CCL-2) was sub-cultured into the culture flask for 4 days. The protocols to sub-culture cells followed as in data sheet of cells. The HeLa cells were harvested and seeded onto TiO_2 nanorods and nanoparticles in a petri dish. The HeLa cells were stained using dapi staining and observed under a fluorescence microscope.

Results and Discussion

The characteristics of TiO_2 nanorods and nanoparticles were observed using fields emission scanning electron microscope (FESEM). Figure 1 shows the structures of TiO_2 nanorods and nanoparticles. The diameter of nanorods ranged from 80 to 100 nm while the diameter of nanoparticles was from 25 to 35 nm. The differences in structure were caused by the methods of fabrication. Hydrothermal method is the reaction process of solution in closed system where low temperature and high pressure was applied in order to produce rutile phased TiO_2 (Gao et al. 2012). This method was able to produce nanorods structure as previous study (He et al. 2013). Whereas, spray pyrolysis deposition method is a technique that atomize the solution particle on heated substrates (Patil et al. 2012; Ranga & Dutta 2007). The results left TiO_2 nanoparticles on heated substrates. Annealing process was done to reduce the organic substances and increase crystalinity and porosity (Ranga & Dutta 2007).

The crystallite structure was analyzed using x-ray diffraction (XRD) Bruker D8. Figure 2(a) shows the rutile peak of TiO_2 nanorods using hydrothermal method at [110], [101], [111] and [211] planes for 2θ at 27.35°, 36.06°, 41.21° and 52.29°, respectively. It agrees with database ICDD 00-004-0551 which prove that the sample is rutile. It can be supported from the study by Dai which had stated that hydrothermal method used with acidic solution would get TiO_2 rutile phased. Referring to database ICDD 00-021-1272, Figure 2(b) shows the anatase peaks of TiO_2 nanoparticles by spray method at [101], [004], [200], and [211] planes for 2θ at 25.38°, 37.98°, 47.66° and 54.82°, respectively. Sapizah et al. (2012) found that the TiO_2 nanoparticles was anatase phased.

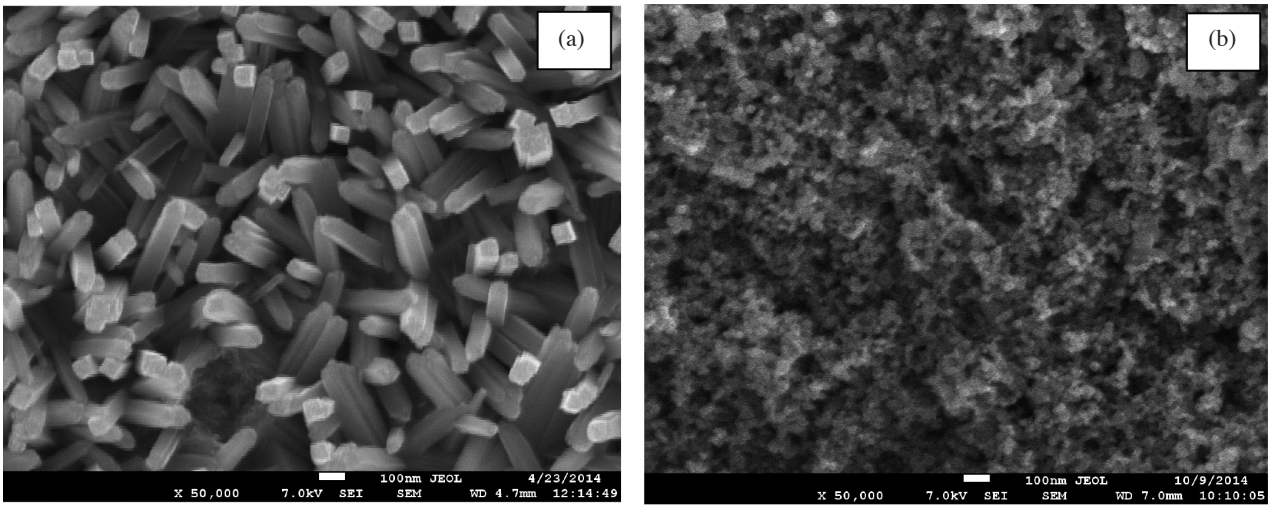

FIGURE 1. FESEM images of (a) TiO_2 nanorods and (b) TiO_2 nanoparticles

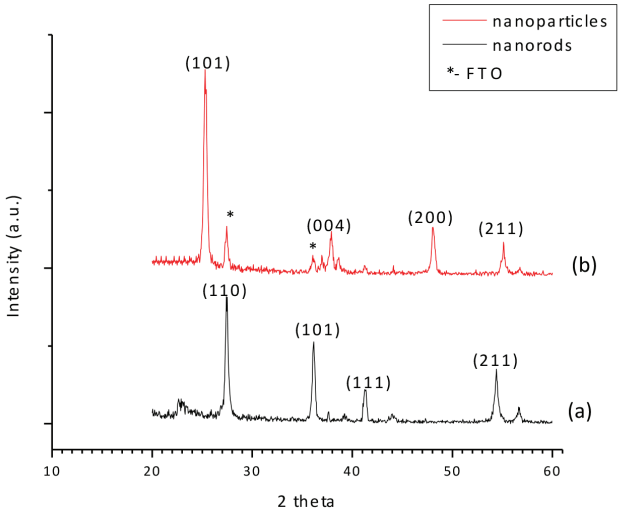

FIGURE 2. XRD pattern of TiO_2 nanorods and nanoparticles that shows rutile and anatase peaks, respectively

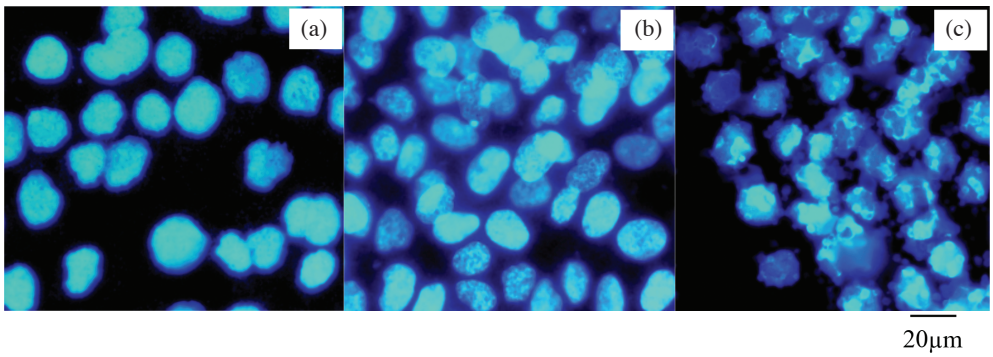

FIGURE 3. HeLa cells grown on (a) control substrate (without TiO_2), (b) on TiO_2 nanorods and (c) on TiO_2 nanoparticles

After that, the TiO_2 nanorods and nanoparticles were used as the substrates to grow HeLa cells. Figure 3 shows the HeLa cells on the surface of TiO_2 nanorods and nanoprticles. The cells were healthy and able to grow on TiO_2 nanorods and nanoparticles surface. TiO_2 nanostructures surface were rough and it supported the cells to attach on them. Kommireddy et al. (2005) reported that roughness of any surface can increase the wettability and thus, it promotes the cells attachment. Moreover, TiO_2 surface is hydrophilic which means that the cells mostly made up from water and made the cells likely to grown on TiO_2 nanostructures surfaces (Khalid et al. 2015).

CONCLUSION

TiO_2 nanorods and nanoparticles were succesfully fabricated from hydrothermal and spray pyrolysis deposition method, respectively. TiO_2 nanorods were in rutile phase and nanoparticles in anatase phase. HeLa cells were successfully grown on TiO_2 nanorods and nanoparticles. Thus, this study has prove that TiO_2 nanostructures were biocompatible to HeLa cells.

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