UNIVERSITI TEKNOLOGI MARA

IN-VITRO TOXICITY EVALUATION OF BISMUTH (III) OXIDE PARTICLES SYNTHESIZED USING DIFFERENT REACTION TEMPERATURES AS PROMISING RADIOGRAPHIC CONTRAST MEDIA

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ABSTRACT

Raise concern about the safety of current contrast media in patients with contraindications to iodinated media spur researchers to find safer options. Bismuth has gained attentions in the development of new contrast media for *in-vivo* imaging due to its high atomic number (Z = 83) which directly possess high X-ray attenuation coefficient, long circulation time in blood and cost effectiveness. However, this hypothetical application is hampered owing to challenges in synthesizing control for *in vivo* stability. This thesis aim to study *in-vitro* cytotoxicity of Bi_2O_3 particles synthesized hydrothermally using different reaction temperatures against iodinated contrast media. The cytotoxicity of Bi_2O_3 particles were assessed using human hepatocytes HeLa [Chang Liver] (ATCC CCL13™) and human embryonic hepatocytes WRL 68 (ATCC CL48™). Bi_2O_3 particles synthesized at 60, 90 and 120 °C were characterized using scanning electron microscope (SEM), transmission electron microscope (TEM) and zetasizer. The cytotoxicity of 100 µg/mL Bi_2O_3 particles in Chang liver and WRL 68 cells was measured using colorimetric cell viability (MTT) assay, intracellular reactive oxygen species (ROS) assay and mRNA expressions of endoplasmic reticulum (ER) stress genes, GRP 78 and CHOP. The characterization results revealed Bi_2O_3 particles synthesized at 60, 90 and 120 °C are rod-shaped with average diameter of 6.164, 6.703 and 7.010 µm, respectively. After 24 hours incubation, the cytotoxicity assays in both cell lines showed Bi_2O_3 particles has reduced cytotoxicity trend with higher reaction temperatures and bigger particles size. Chang liver cells are more susceptible to Bi_2O_3 particles cytotoxicity depicted with higher reduction of treated cells and high level of ROS while WRL 68 cells has higher resistant as only Bi_2O_3 particles synthesized at 60 °C was observed to be cytotoxic and all Bi_2O_3 particles induced low level of ROS. Bi_2O_3 particles showed acute cytotoxicity in both cell lines as the viability of treated cells increased with prolonged incubation time. Following to two different responses to cytotoxicity of Bi_2O_3 particles, GRP 78 and CHOP genes were expressed at low level in Chang liver cells to allow adaption for cell survival. Treated WRL 68 cells showed upregulations of GRP 78 and CHOP genes in decreasing cytotoxicity trend of Bi_2O_3 particles with higher reaction temperatures. In comparison to clinically-used iodine, Bi_2O_3 particles synthesized at 120 °C showed lower cytotoxic effect and suggest good biocompatibility as new contrast media.

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CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

Over the past decades, researchers focused on the development of nano and micro-sized particles prior to biological and biomedical applications. The efforts made by health professionals in the globalisation era to combat diseases lead to the findings of safer, effective, cheaper and less toxic alternative medicine. Small-sized particles in medicine may serve in five main areas; novel therapeutics and delivery system, analytic tools, nanomaterials and nanodevices, nanoimaging and clinical and toxicological. (Roy, Gaur, Jain, Bhattacharya, & Rani, 2013). The application of nanotechnology in medicine allows highly specific medical interventions for diagnosing, treating and preventing disease, relieving pain and preserving human health at molecular scale. Nanomaterials are known to be extremely small and possessed high surface volume ratio give benefits to the physicochemical properties in contrast to its bulk materials (Nath & Banerjee, 2013). Surface modifications of nanomaterials can be made to enhance its usage and as stabilizing agent. Nevertheless, this unique physicochemical properties of small-scale particles may exert adverse effects of its great importance (Bergs et al., 2015; Bernal et al., 2014). Nanotoxicology is a branch of study focused on engineered nanoparticles and its adverse side effect in living organisms (Ahmad et al., 2012; Oberdörster, 2010).

Under X-ray radiation owing to the inherent contrast, electron-dense bone structures can be superbly visualized in comparison to more permeable soft tissues (Mongan et al., 2012). A contrast media is a foreign material administered intravenously into patient to increase radiographer's ability to visualize anatomical region of interest of various soft tissues which cannot be differentiate by unenhanced X-ray imaging. Currently, 1,3,5-triiodobenzene made up of water-soluble iodinated molecules are routinely used as contrast media in current diagnostic clinical settings for *in-vivo* contrast enhancement. However, efficacy of this iodinated agents are hampered by limitations such as; (i) rapidly excreted by kidneys due to its low molecular weight, thus causing short circulation time in *in-vivo*, (ii) low K-edge value (33 KeV) not optimal for X-ray attenuation hence giving low contrast efficacy, (iii)