Abstract

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EVALUATION OF PARTIAL VOLUME EFFECT CORRECTION TECHNIQUE USING ADAPTIVE REGION GROWING TEMPLATE FOR SPECT QUANTIFICATION

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Introduction: Partial volume effect (PVE) occurs due to limited spatial resolution of the gamma camera which degrades the quantitative accuracy in SPECT imaging.

Objective: The aim of this study was to develop and evaluate the template based adaptive region growing method to correct for PVE in SPECT images of small target objects with background activities.

Materials and Method: Using a validated Geant4 Application of Tomography Emission (GATE) simulation model, SPECT images of rectangular phantom containing target objects of various sizes ranged from 1.44 mL to 10.1 mL surrounded by radioactivity with target to background activity concentration (T/B) ratio of 1.25, 2.5 and 5.0 were simulated. Developed PVE correction was applied on each simulated SPECT data. Then the SPECT slices were reconstructed using filtered back-projection method. Image contrast, total counts, volume of target object and activity concentration were quantified using SPECT projections with and without PVE correction. The results were then compared.

Results: For all T/B, the edges of hot region of interest (ROI) were enhanced with PVE correction. Improvement in contrast of target objects were observed for SPECT images with PVE correction. For SPECT slices with high background activity (T/B=1.25), the counts in target objects increased up to 40% after PVE correction for object size larger than 6.3 mL. For object size less than 6.3 mL, the counts in target objects increased up to 55% after PVE correction. For SPECT images with low background activity, the counts in the target objects increased more than 50% after PVE correction for all target objects. The agreement between simulated volume and estimated volume of target objects with PVE correction (R^2 = 0.3715) was better compared to without PVE correction (R^2 = 0.1077). Similarly, the agreement between simulated radioactivity concentration and estimated radioactivity concentration was better with PVE correction (R^2 = 0.9512) compared to without PVE correction (R^2 = 0.9444).

Conclusion: The developed PVE correction technique improved the accuracy of SPECT quantification. The initiative to correct for PVE gives a positive outcome by enhancing the edges of region of interest and eliminating the background counts effectively for SPECT images with poor contrast.

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GENETIC RELATIONSHIPS OF SIX ORANG ASLI SUBGROUPS BASED ON Y-STR AND FULL MITOCHONDRIAL DNA SEQUENCE

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Introduction: Orang Asli (OA) populations are the earliest inhabitant in Peninsular Malaysia since their arrival 60,000 years ago. They express similar phenotypes and show close genetic affinity with African populations. Unfortunately, these relic populations are now suffering severe genetic drift due to the current development and environmental degradation. Several OA subgroups continue to have only slow, if any, population growth. Thus, it is crucial to understand and preserve the precious genetic information of these valuable populations. Y chromosome is the smallest human genome and is purely inherited from father to son throughout generations. On the other hand, mitochondrial DNA (mtDNA) is an independent genome which is exclusively inherited from mother to offspring. These two genetic markers could provide genealogical and phylogeographic resolution of a population.

Objectives: The present study is the first to determine the molecular polymorphisms of Y chromosome and full mitochondrial DNA sequence for Orang Asli subgroups: Kensiu, Lanoh, Bateq, Semai, Che Wong and Orang Kanaq. The generated datasets is significant in unravelling the theory of origins and migration patterns of OA population as well as to date the first arrival of modern human in Peninsular Malaysia.

Materials and Methods: This research involved six OA subgroups; a total of 90 male individuals were recruited in order to establish the 17 Y Chromosome Short Tandem Repeats (Y-STRs) haplotype data. For the maternal lineage, a total of 120 mitochondrial DNA genomes (mtGenome) were

analysed using Next Generation Sequencing by Ion Torrent Personal Genome Machine system.

Results and Discussions: In total, 52 Y-STR haplotypes were observed and the calculated haplotype diversity was 0.9793. Haplogroup O was recorded with the highest frequency of 42.2%, followed by haplogroup N with 16.7%. The Time to the Most Recent Common Ancestor (TMRCA) for paternal lineage was dated around 35,000 years ago. MtGenome analyses have generated 113 haplotypes. The gene diversity for Kensiu, Orang Kanaq and Semai were 1.0000, which indicated unique profile of each individual within the subgroups. Macro-haplogroup M was the most frequent mtDNA haplogroup with 58.3% among all six OA subgroups, followed by major-haplogroup R with 37.5%. In contrast, super-haplogroup N was only present in Kensiu (10.0%) and Semai (11.5%). Based on the TMRCA, the maternal lineage of OA was dated approximately 58,000 years ago. Generally, the multi-dimensional scaling, principal coordinates analysis and phylogenetic analyses produced similar distribution pattern of all OA subgroups. Both Semang subgroups (Kensiu and Lanoh) might share common paternal and maternal ancestors as they clustered together. Semai was plotted closer to Bateq while Che Wong and Orang Kanaq were separated from other OA subgroups. Comparative analyses with the global population depicted Kensiu, Lanoh and Bateq was grouped together with other ancient populations: African Aborigines, Indian and Australian Aborigines. The Senoi was positioned in between the African Aborigines/Indian/Australian Aborigines and Chinese Han/Austroasiatic speakers inferring experience of genetic admixture. Orang Kanaq, the only Proto-Malay subgroup in this study, showed neighbouring connection with the Malays in Peninsular Malaysia.

Conclusion: This study supported the 'Out of Africa' migration theory and proven that OA populations in Peninsular Malaysia are the descendants of this migration wave. OA is also one of the oldest populations in the world surviving to date.

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EVALUATION OF WATER QUALITY AND APPLICATION OF VOLTAMMETRY FOR HEAVY METALS ANALYSIS OF RIVERS AND TUBEWELLS IN KELANTAN FOR INDUSTRIAL AND NON-INDUSTRIAL AREAS

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Introduction: Industrialisation has long been accepted as a hallmark of civilisation due to its substantial contributions to economic growth and human welfare but it carries with it inevitable costs and problems in term of environmental pollution. Significant adverse effects are elicited on the environment when industrial wastes are discharged directly or indirectly into the environment. The discharge of untreated industrial waste into rivers alter the physical, chemical and biological characteristic and lastly deteriorated the quality of water.

Objectives: The present study is aimed at evaluating the water quality index (WQI) and heavy metals for rivers and tube wells in the study areas of Pengkalan Chepa and Tumpat, Kelantan, Malaysia.

Materials and Methods: These have been determined by collecting 5 water samples each from rivers in industrial area, rivers in non-industrial area, tube wells in industrial area and tube wells in non-industrial area during dry and wet seasons.

Results: The WQI was calculated using DOE-WQI based on the following parameters: pH (5.49–6.98), temperature (28.5–33.20), dissolve oxygen (1.30–7.20) mg/L, ammonical nitrogen (0.00–0.60) mg/L, suspended solid (1.00–32.00) mg/L, chemical oxygen demand (4–1650) mg/L and biochemical oxygen demand (0.10–1.30) mg/L. The results of water quality index indicated that water samples of rivers and tube wells in industrial and non-industrial areas for both dry and wet seasons are characterised by slightly polluted and classified into class II and class III (54–84) based on Interim National Water Quality Standard (INWQS) criteria. The heavy metals, cadmium (0.120) µg/L, copper (15.142–107.600) µg/L, lead (3.160–46.792) µg/L and zinc (1.757–327.996) µg/L were determined simultaneously by using Different Pulse Anodic Stripping Voltammetry Technique (DPASV).

Conclusion: Heavy metals elements (Cd, Cu, Zn) in all water samples showed that the levels are under the permissible limits as suggested by World Health Organization (WHO) and Ministry of Health Malaysia (MOH), however for element Pb, the values obtained at certain sampling locations is more than 10 µg/L.

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DOSIMETRIC CHARACTERISATION OF GAFCHROMIC™ EBT3 FILMS FOR NANOPARTICLES-ENHANCED PHOTON, ELECTRON, HIGH DOSE RATE 192-IR AND PROTON RADIOTHERAPY

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Introduction: Investigators have identified promising capabilities of high atomic number (Z) metallic nanoparticles (NPs) in enhancing radio-therapeutic effects. Principally, the bombarding of NPs with radiotherapy beams increases the dose deposition within the targeted volume by increasing the probability of radiation interaction and intensifying the generation of radiation-induced products such as secondary electrons and X-rays. This phenomenon is called physical dose enhancement (DE).

Objectives: This work aims to dosimetrically investigate the contribution of radiation-induced products generated by different NPs towards the physical DE, using radiochromic films (RCFs).

Materials and Methods: Optimisation of physical DE detection method was first pursued by characterising standard and delaminated GAFCHROMIC™ EBT3 RCFs (1.0 cm × 1.2 cm) using two types of densitometers (flatbed scanner and ultraviolet-visible spectrophotometer). The selected EBT3 RCFs were then immersed separately into two mediums (NPs with Phosphate Buffer Saline (NPs + PBS) and NPs with White Soft Paraffin (NPs + WSP)) of 0.5 mL, loaded with either gold (AuNPs), bismuth (BiNRs), or superparamagnetic iron oxide (SPIONs)) at 10 mM concentration. Irradiations were conducted at a single dose of 400 cGy using four different types of radiotherapy modalities: photon beams (6 MV, 10 MV), electron beams (6 MeV, 12 MeV), High Dose Rate (HDR) Iridium-192 brachytherapy source (0.38 MeV), and a proton beam (150 MeV). Experimental dose enhancement factors (DEFs) were then measured using the optical density data obtained from the selected densitometer type, and the results were compared to an analytically-calculated theoretical DEF.

Results: After dosimetric evaluation, the standard EBT3 RCFs were selected over the delaminated EBT3 RCFs for physical DE detection due to its immersion ability and significantly lower net total uncertainty. In terms of the densitometer, the flatbed scanner was chosen over the spectrophotometer for its simplicity and sufficiently accurate measurements. Characterisation of the selected dosimetry system for all immersion setup was found to yield a net total uncertainty of ± 5.00%, which is within the accuracy requirement of dose delivery in radiotherapy. However, evidence from the experimental work suggested that no significant DEFs were able to be recorded as the signals were less than the uncertainty and the experimental DEFs were statistically indifferent from the control groups across all modalities and NP types ($P > 0.05$). NP-type dependencies were also unable to be observed in the experimental DEFs as opposed to the theoretically-calculated DEFs.

Conclusion: This study demonstrated that under the investigational conditions, no significant physical DE signals were able to be detected by the standard EBT3 RCFs. Thus, the enhancement of cancer cell deaths shown in published biological studies with metallic NPs and radiotherapy could be attributed mainly to the underlying chemical-biological interactions. Further researches using different experimental setups and computational simulations should be performed to provide better insights on the quantification of physical DE.

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BISMUTH OXIDE NANORODS AS RADIOSENSITIZER FOR RADIOTHERAPY

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***Introduction*:** Metallic nanoparticles such as gold, platinum, iron and silver have been of interest as radiosensitiser to enhance radiotherapy treatment. The use of high atomic number material such as bismuth nanoparticles (Z = 83) will increase the radiation interaction and absorbed dose which leads to cancer cell death.

***Objectives*:** In this study, hydrothermal technique was implemented to synthesize bismuth oxide nanorods (Bi_2O_3-NR) of sizes 60 nm, 70 nm, 80 nm and 90 nm. The properties of Bi_2O_3-NR were then characterised to determine the phase presence, crystallinity morphology, elemental presence and size of the nanoparticles. Then the cytotoxicity test was run to reveal that the Bi_2O_3-NR of concentrations 0.05, 0.25, and 0.5 µMol/L are safe to be used as radiosensitiser. Cell survival curves were plotted after breast cancer cell line (MCF-7) and cervical cancer cell line (HeLa) were incubated with Bi_2O_3-NR and irradiated with megavoltage photon and electron beams. Sensitisation enhancement ratio (SER) analysis from cell survival curves was generated to analyse optimum radiosensitisation of Bi_2O_3-NR. Finally, the presence of Bi_2O_3-NR that generate reactive oxygen species (ROS) was investigated in facilitates DNA damage.

***Materials and Methods*:** The Bi_2O_3-NR were synthesised using a hydrothermal method with different weights of bismuth(III) nitrate pentahydrate ($Bi(NO_3)_3 \cdot 5H_2O$). The particle size, and crystal structure of as-synthesised particles were investigated by X-ray diffraction (XRD), Field Emission Scanning Electron Microscope (FESEM), Energy-dispersive X-ray spectroscopy (EDX) and Fourier transform Infra-Red (FTIR). Using MCF-

7 and HeLa cell lines, the Bi_2O_3-NR with concentration 0.05 µMol/L, 0.25 µMol/L and 0.5 µMol/L was incubated for 24 h, 48 h and 72 h. Then the cytotoxicity level was verified using PrestoBlue. To evaluate the effects of Bi_2O_3-NR on the radiosensitisation effects, MCF-7 and HeLa cell was incubated with Bi_2O_3-NR and placed under Linear accelerator (LINAC) to expose with 6 MV and 10 MV photon beam. The 6 MeV and 12 MeV electron beam also was used to investigate effectiveness of Bi_2O_3-NR in kill cancer cells. Then the data was extrapolated using linear quadratic model and SER calculation to analyses the radiosensitisation of Bi_2O_3-NR. Finally, this work used 2',7'-dichlorofluorescein diacetate (DCF-DA) to assess the activity of ROS in the presence of Bi_2O_3-NR.

***Results*:** In this study, the Bi_2O_3-NR was successfully synthesised using hydrothermal method. Different weights of Bi(NO3)3•5H2O synthesised different size of Bi_2O_3-NR with the average size of nanoparticles is 60 nm, 70 nm, 80 nm and 90nm. The nanoparticles are of a pure monoclinic Bi_2O_3 phase with rods shape. The cytotoxicity test confirmed the concentration 0.05, 0.25 µMol/L and 0.5 µMol/L of Bi_2O_3-NR is safe to use as radiosensitiser. In this study, Bi_2O_3-NR of concentration 0.5 µMol/L is the most optimum concentration in killing cancer cell by generating high SER value that potential to destroy cancer cells. The smallest size of Bi_2O_3-NR (60 nm) has the huge potential to destroy cancer cells by generate secondary electron. Comparison between photon and electron beam, the data showed the Bi_2O_3-NR is more effective when irradiated with photon beam. This was support by ROS production is increased when Bi_2O_3-NR was exposed with photon beam and enhanced radiotherapy effect by causing damages to DNA and cellular organelles.

***Conclusion*:** From this study, we successful synthesised Bi_2O_3-NR used hydrothermal method. All sizes of Bi_2O_3-NR are not toxic to MCF-7 and HeLa when exposed with high concentration (0.5 µMol/L). This study analysed the highest radiosensitisation by Bi_2O_3-NR at 10 MV photon beam with concentration 0.5 µMol/L. The Bi_2O_3-NR induce more ROS at dose 8 Gy, generated from a medical LINAC. Therefore, this study concluded the Bi_2O_3-NR is an ideal radiosensitiser to enhance radiotherapy treatment.

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CHARACTERISATION OF PHENOTYPES OF MONOCYTIC MICROPARTICLES (MMP) DERIVED FROM CD14$^+$ MONOCYTE AND CD16$^+$ MONOCYTE SUBSETS AND THEIR EFFECTS ON COAGULATION AND CYTOKINE SECRETIONS

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***Introduction*:** Monocytic microparticles (mMP) are microvesicles derived from plasma membrane of monocytes ranging between 100 nm–1000 nm in diameter which are released in response to cellular activation, apoptosis or during certain pathological conditions. Monocytic MP play important roles in inflammation, blood coagulation and endothelial cell function, although the exact mechanism involved remains unclear.

Objectives: The aim of this study was to assess cell surface antigen expression on mMP derived from different monocyte populations, their potential role in blood coagulation and cytokine secretions of monocytes in the presence of mMP were also assessed by ELISA.

Materials and Methods: In this study, peripheral blood mononuclear cells (PBMC), whole monocytes, CD14$^+$ and CD16$^+$ monocytes were isolated. To induce mMP secretion, different monocyte populations were stimulated with 1 µg/mL of lipopolysaccharide (LPS), followed by mMP purification by several centrifugation steps.

Results: This study has shown that mMP derived from whole monocytes, CD14$^+$ monocytes and CD16$^+$ monocytes expressed CD14 and CD16 surface antigens. Interestingly, our finding has also demonstrated that mMP derived from CD14$^+$ monocytes and CD16$^+$ monocytes did not alter coagulation by prothrombin time (PT) although express high CD142. In addition, LPS-stimulated monocytes-mMP co-culture resulted in pro-inflammatory cytokine secretions, tumour necrosis factor (TNF)-α and interleukin (IL)-6 compared to their unstimulated counterparts.

Conclusion: Taken together, mMP derived from different monocyte subsets were shown to inherit their parent cell antigen expression by expressing Annexin V$^+$/CD14$^+$ and Annexin V$^+$/CD16$^+$, involved in blood coagulation and capable of inducing pro-inflammatory cytokine secretion by monocytes.

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EVALUATION OF PHYSICOCHEMICAL PROPERTIES AND REPEATED DOSE 28-DAYS ORAL TOXICITY OF AGED TUALANG HONEY IN SPRAGUE DAWLEY RATS

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Introduction: Aged tualang honey (TH) is still becoming a choice, especially by the old folks in Malaysia due to cultural beliefs that aged honey contains higher nutritional values, yet more beneficial. However, the safety of consuming aged TH (ATH) is questionable due to the fact that it presents higher 5-hydroxymethylfurfural (HMF) concentration.

Objectives: The study focuses on evaluation of the effects of four years stored ATH on the physicochemical properties including HMF concentrations (Study I) and ultimately on the repeated dose 28-days oral toxicity study of ATH (Study II).

Materials and Methods: In Study I, the levels of pH, free acid, lactone, total acidity, moisture content and HMF were determined using standard procedures. While in Study II, the study was conducted by treating Sprague Dawley rats with control, 200, 1000, 2000 mg/kg/day ATH or 2000 mg/kg/day fresh TH for a duration of 28 days. A substantial number of parameters were assessed throughout this study.

Results: In Study I, the results obtained positively showed a significant decrease in the pH value of ATH. Differently, free acid, lactone, total acidity, moisture content and HMF concentrations were highly increased, exceeding the international standard values set for honey. Meanwhile, the results acquired from Study II indicated that ATH at all dose were safe for rats. Nevertheless, at the dose of 1000 mg/kg, there were significantly increased in the creatinine and oestradiol levels of female rats. Yet, the increment in both parameters were still within the normal range of rats. Besides that, there were reduction in kidney's weight of the females treated with ATH at the dose of 200 and 1000 mg/kg. The histological findings later disclosed that there were reductions of Bowman's space and poorly defined glomerular boundary for the females treated with 1000 mg/kg ATH. In contrast, all parameters of male rats were statistically unaffected by ATH.

Conclusion: Taking all the cumulative data together, this study suggests that the prolonged storage period of ATH allows alteration in its physicochemical properties, mostly outside the range permitted by the international standard. The administration of ATH did not resulted in any definite toxicity effect apart from significantly increased in the creatinine and oestradiol values, the reduction of Bowman's space and poorly defined glomerular boundary in female rats. Thus, the consumption of ATH is generally safe but it must be taken with precaution.

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