EVALUATION OF TUMOUR CELLS DAMAGE FOLLOWING RADIOTHERAPY BY TC-99M PERTECHNETATE

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ABSTRACT

Radiotherapy has become the most important modality in treating cancer with approximately 50% of cancer patient undergo the treatment. However, more improvement to the radiotherapy treatment efficacy is required to deprive cancer. Assessment of tumor progress during treatment is important to accommodate the changes that occur during the fractionation course. The objective of this study is to assess tumor cell damage after external beam radiotherapy by using technetium-99m pertechnetate ($^{99m}TcO_4^-$) as a tracer. In this study, HeLa cells were irradiated with 6 MV photon beam with different radiation dose ranging from 0.5 Gy to 10 Gy. The irradiated cells were recultured in 6-well plates and incubated for 10 days. After that, 2 mCi of $^{99m}TcO_4^-$ were prescribed to each cell colonies. The viable cells were separated from the rest and measured for $^{99m}TcO_4^-$ uptake using single head gamma camera with LEHR collimation. As results, the cells survival fractions clearly indicate diminishing effect to the cells at higher dose of irradiation. Good correlation were observed between $^{99m}TcO_4$ uptake and survival fraction for cells irradiated at lower dose and less significant correlation were indicated at higher dose. In conclusion, there is potential for the efficacy of external beam radiotherapy in treating cancer to be assessed by using radioisotope as a non-invasive tracer. In this case, technetium-99m pertechnetate ($^{99m}TcO_4$) could be attached to the specific antibody so that better correlation between the cells uptake and possible cell damages could be observed.

ABSTRAK

Radioterapi telah menjadi modaliti utama dalam merawat kaser, di mana lebih 50% pesakit kanser melalui kaedah rawatan ini. Namun yang demikian, masih banyak ruang penambahbaikan perlu dibuat untuk meningkatkan keberkesanan radioterapi dalam merawat kanser. Penilaian terhadap perubahan tumor adalah penting ketika membuat sebarang perubahan sepanjang prosedur rawatan berlangsung. Objektif kajian ini adalah untuk menilai kerosakan terhadap sel kanser akibat radioterapi dengan menggunakan technetium-99m pertechnetate ($^{99m}TcO_4^-$) sebagai penanda. Sel HeLa telah didedahkan dengan pancaran foton 6 MV, yang mempunyai dos radiasi antara 0.5 hingga 10 Gy. Sel HeLa tersebut kemudiannya dikultur semula dan diinkubasi selama 10 hari. Seterusnya sebanyak 2 mCi $^{99m}TcO_4^-$ telah dimasukkan kedalam setiap bekas sel. Sel yang hidup diasingkan untuk diukur kandungan $^{99m}TcO_4^-$ menggunakan kamera gamma berkolimasi LEHR. Hasil kajian jelas menunjukkan bahawa pecahan survival sel berkurangan apabila dos radiasi meningkat. Kolerasi antara survival sel dan penyerapan $^{99m}TcO_4^-$ adalah baik bagi dos rendah, namun kolerasi tersebut menurun apabila dos

meningkat. Konlusinya, radioisotop mempunyai potensi untuk digunakan sebagai penanda bagi melihat keberkesanan radioterapi secara tidak invasif. Dalam kes ini, technetium-99m pertechnetate ($^{99m}TcO_4^-$) boleh disambung dengan antibody yang spesifik bagi meningkatkan kolerasi antara penyerapan $^{99m}TcO_4^-$ ke dalam sel dan kerosakan sel akibat radioterapi.

Keywords: technetium-99m pertechnetate, molecular imaging, radiotherapy

INTRODUCTION

Cancers have become a main prominent cause of deaths among men and women around the world. According to GLOBOCAN (2012), an estimated 14.1 million new cancer cases and 8.2 million cancer-related deaths happened in 2012, compared with 12.7 million of cases and 7.6 million of death in 2008. Prevalence assessments for 2012 revealed that there were 32.6 million people (over the age of 15 years) alive who had a cancer diagnosed in the previous five years (WHO, 2014).

Debate continues about the best treatment to handle malignancy. Although recombination of treatment becomes most popular option to handle cancer, external beam radiotherapy is still a vital choice, especially if the tumour is spreading within small sizes. The patient undergoes radiotherapy require post therapy assessment to investigate the tumour's progress. General X-rays, Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are commonly used for this purpose. But these modalities could assess response only after patient had completed the all fractions of treatments because anatomical changed may occur only after treatment. Efficacy of external beam radiotherapy could not be displayed with these diagnostic techniques during early of treatment.

If the radiotherapy efficacy can be assessed earlier by assessing the tumour cells damage via molecular imaging, treatment modification can be done and will possibly increase in the patient survival rate. The molecular marker such as cell death can potentially be used to predict tumour cell damage and its relationship with radiopharmaceutical uptake could be an indicator. Cell death was an essential biological processed for eliminating abundant and unwanted cells during embryonic development, growth, differentiation and maintenance of tissue homeostasis. There are few types of cell death, such as apoptosis, necrosis and mitotic (Verheij, 2008). Studies suggested that apoptosis is a major form of cell death following radiotherapy (Yang et al., 2012). To date, Annexin V-based tracers are the most frequently used agents for in vitro detection and quantification of apoptotic cells (Khoda et al., 2012). However, more applicable technique is required to assess tumour cell death in vivo and using nuclear medicine technique seems a good option.

The purpose of this study is to assess tumor cell damage after external beam therapy by using technetium-99m pertechnetate ($^{99m}TcO_4^-$) as a tracer. Correlation between irradiation dose and $^{99m}TcO_4^-$ uptake by HeLa cells were investigated.

MATERIALS AND METHODS

HeLa cell lines preparation.

HeLa (ATCC® CCL-2™) cell lines were prepared in Dulbecco's Modified Eagle's Medium (DMEM) Complete Media, which was supplemented with 10% FBS and a 100 unit/mL penicillin-streptomycin. All cells were

incubated at 37°C and 5% CO_2 humidified atmosphere. The cells were grown until confluence and harvested using 0.25% Trypsin-EDTA.

HeLa cells irradiation setup.

Solid water phantoms were organized with the thickness 13.5 cm on LINAC table couch. Cells samples were placed at the center of the beam on top of the solid water phantoms and then covered with 1.5 cm bolus. The samples were irradiated with different radiation dose (0.5 Gy to 10 Gy) using 6 MV photon beam at 100 cm SSD and 10 cm x 10 cm field size. The cell samples were counted immediately to see the viability right after irradiation and then recultured for 10 days for the cells to form colony (clonogenic assay). After 10 days, $^{99m}TcO_4^-$ uptake study was conducted on cell colony and the uptake were compared with the cells colony formed.

Cell viability measurement.

The viability of irradiated cell samples was measured using tryphan blue exclusion methods. The cells were stained using tryphan blue and counted on hemacytometer under microscope. The numbers of viable cells were counted. The counting of viable cells versus non-viable cells were made possible by using trypan as the non-viable cell cytoplasm will look darker compared to viable cell, which have clear cytoplasm after treated with this assay (Strober, 2001).

Clonogenic cell staining.

Cell samples that have been incubated for 10 days were rinsed off of their cell media using 0.5 ml of PBS. Cells were fixed using 0.5 ml ice cold methanol for 15 minutes. Crystal violet were used to stain the cells and after staining process for 30 minutes, the cells were rinsed gently using tap water, then let to dry completely. The visible cell colonies were counted using microscope and analyzed in form of cell survival fraction data using OriginPro 7.5 software.

$^{99m}TcO_4^-$ uptake measurement.

0.2 mCi of $^{99m}TcO_4^-$ in form of sodium pertechnetate were administered into each samples. The samples were incubated again for another 30 minutes to allow $^{99m}TcO_4^-$ uptake by cells. After 30 minutes, the cells were rinsed with 0.5 ml PBS. The cells were then make into suspension using Trypsin EDTA and were centrifuges at 1500 rpm for 5 minutes. The centrifuged cells were scanned using using gamma camera equipped with LEHR collimator. $^{99m}TcO_4^-$ uptake measurements were performed with the detector of gamma camera at 10 cm distance to the cell samples. The count reading was measured for 100 second using 20% window at 140 keV. The percentage $^{99m}TcO_4^-$ uptake was calculated and graph $^{99m}TcO_4^-$ percentage uptake versus irradiation dose was plotted.

RESULTS AND DISCUSSIONS

In this work, HeLa cells were used as an in-vitro model to identify tumor cell damage after radiotherapy. Figure 1 show the number of viable cells which were counted immediately after irradiation with different dose of 6 MV photon beam. Figure 1 clearly shows that the number of viable cells correlates inversely with radiation dose. The loss of reproductive capacity after radiation was associated with early cell death, which may represent the effectiveness of radiotherapy techniques used in the treatment. Joiner and Kogel (2009) pointed that the potential reason of the early cell death was resulting from activation of pathways in response to the initial cellular damaged caused by irradiation (Joiner et al., 2009).

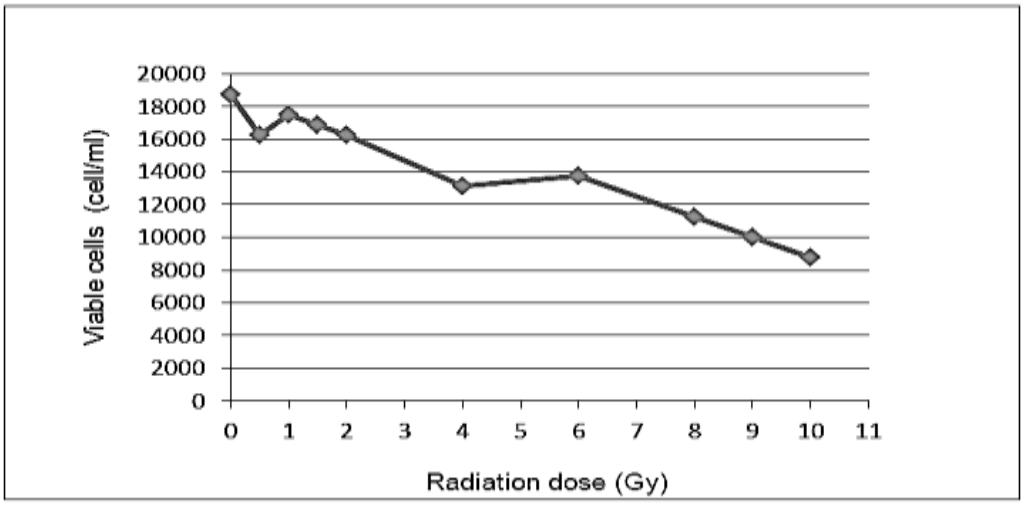

Figure 1. The viable cells (cell/ml) measured immediately after versus radiation dose (Gy)

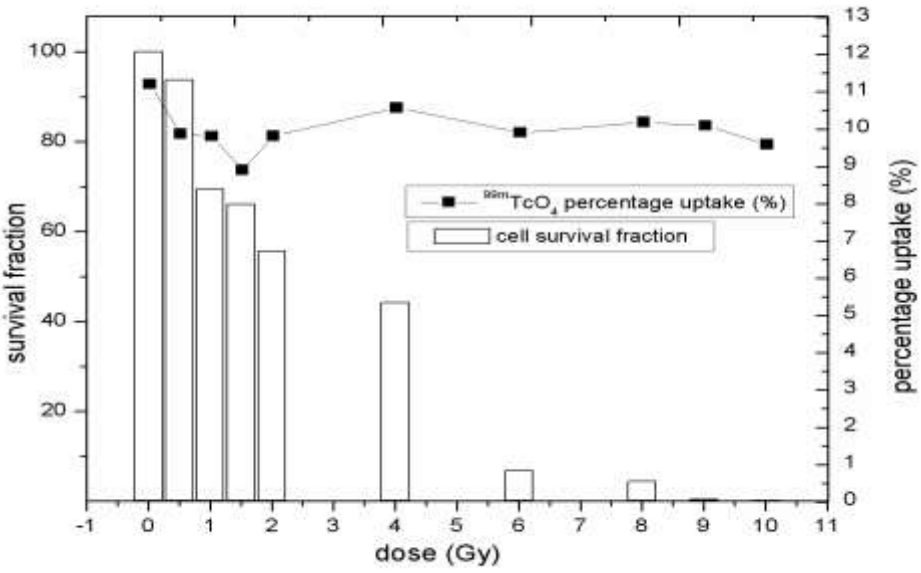

Figure 2. Correlation between percentage $^{99m}TcO_4^-$ uptake and survival fraction of the HeLa cells.

The cell damage also has been assessed using colony forming assay. The cells that form colony were tested for $^{99m}TcO_4^-$ uptake which was employed as indicator to assess cell damage in response to the radiation dose. Based on Figure 2, the cell survival fraction clearly shows decrement as the radiation dose increases. However, $^{99m}TcO_4^-$ uptake among the irradiated HeLa cells does not show any significant correlation with survival fraction. This result contradicted with another similar study conducted by Tabar et al. (2011) and Liang et al. (2008), which used radiopharmaceuticals in evaluating chemotherapy efficacy. Both of these studies shows inverse relation between their respective radiopharmaceutical uptake and cell's apoptotic response. But it is worth to point out that their study used the radiopharmaceuticals that are already tagged with carriers that can selectively be absorbed by their respective cell samples. So we assume that the reason for our contradicting results with these

studies is because of unsuitable pairing between radiopharmaceutical and target cells. Each radiopharmaceuticals have their own affinity with different type of cells and tissues. $^{99m}TcO_4^-$ are already well known to be used clinically for thyroid imaging, parathyroid imaging and Meckel's scan, so choosing the suitable pairing of radiopharmaceutical and targeted cells is crucial. In this case, $^{99m}TcO_4^-$ uptake by cells could be optimized with specific antibody and targeting agent.

CONCLUSION

In conclusion, there is potential for the efficacy of external beam radiotherapy in treating cancer to be assessed by using radioisotope as a non-invasive tracer. In this case, technetium-99m pertechnetate ($^{99m}TcO_4$) could be attached to the specific antibody so that better correlation between the cells uptake and possible cell damages could be observed. Further improvised study are advised so that we can understand more about the relation between cell damages due to radiotherapy, and its effect on intercellular uptake of radiopharmaceuticals.

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INTERCELLULAR UPTAKE OF TECHNETIUM-99M PERTECHNETATE BY DIFFERENT TYPES OF CELL LINES

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ABSTRACT

The purpose of this study is to determine the technetium-99m pertechnetate ($^{99m}TcO_4$) intercellular uptake by different types of cell lines. HeLa, human fetal osteoblast (hFOB), glial and glioma cell lines grown in 6-wells culture plates were incubated with $^{99m}TcO_4$ of activity of 200, 400, 600, 800 and 1000 μCi for 30 minutes at 37°C and 5% CO_2 humidified atmosphere. After incubation, the cells were washed 3 times with phosphate buffer saline to remove the extracellular traces of $^{99m}TcO_4$. Measurements of the intercellular $^{99m}TcO_4$ radioactivity were performed using single head gamma camera and the percentage uptake of the $^{99m}TcO_4$ into the cells was calculated. The intercellular uptake of $^{99m}TcO_4$ was found to be inversely correlate to the radioactivity. HeLa cell shows the highest uptake followed by hFOB, glial and glioma cell lines. Comparison of uptake between normal and cancer cells present indistinguishable results. The findings of this study suggest that the intercellular uptake of $^{99m}TcO_4$ is highly dependent on the type of cells despite no significant different of uptake was found between normal and cancer cell lines. The level of radioactivity is also an important determinant factor that influence the uptake of $^{99m}TcO_4$ into the cells. This study will be the first precedent toward understanding the cellular characteristic and pharmacokinetic of non-invasive imaging tracer for future molecular imaging and therapy.

ABSTRAK

Kajian ini bertujuan untuk mengenal pasti kadar penyerapan intersel technetium-99m pertechnetate ($^{99m}TcO_4$) oleh jenis sel yang berbeza. Kumpulan sel HeLa, sel human fetal osteoblast (hFOB), sel glial dan sel glioma dikultur dalam piring kultur dan diinkubasi bersama 200, 400, 600, 800 and 1000 μCi $^{99m}TcO_4$ selama 30 minit (37°C, kelembapan atmosfera CO_2 5%). Selepas proses inkubasi, sel dibasuh dengan phosphate buffer saline untuk membuang sisa-sisa ekstrasel $^{99m}TcO_4$. Pengukuran radioaktiviti $^{99m}TcO_4$ intersel dilakukan menggunakan kamera gamma, kemudian peratusan serapan $^{99m}TcO_4$ oleh sel-sel dikira. Hasil kajian menunjukkan kadar serapan intersel $^{99m}TcO_4$ berkadar songsang dengan radioaktiviti. Sel HeLa menunjukkan kadar serapan yang lebih tinggi berbanding sel hFOB, diikuti dengan sel glial dan sel glioma. Didapati tiada perbezaan kadar serapan antara sel kanser dan sel sihat. Konklusinya kajian ini menunjukkan bahawa kemungkinan kadar serapan intersel terhadap $^{99m}TcO_4$ sangat bergantung kepada jenis sel, namun tiada perbezaan signifikan ditunjukkan apabila sel sihat dan sel kanser dibandingkan. Paras radioaktif juga merupakan factor yang penting dalam mempengaruhi serapan $^{99m}TcO_4$ oleh sel.

Keywords: Technetium-99m-pertechnetate, In-vitro, molecular imaging

INTRODUCTION

Cancer detection through variety of medical imaging procedures such as scanning using magnetic resonance imaging (MRI), computed tomography (CT) scanner, single photon emission computed tomography (SPECT) and positron emission tomography (PET) provide different information and details on the degree of malignancy [1]. Screening cancer by employing radionuclide and appropriate radiotracer to identify diseases not only detect the location of the disease but also the physiology of the abnormality that can significantly impact the cancer patient management [2]. The details of the diseases at cellular level are vital for the accurate diagnosis and treatment prescription [3]. Radionuclides such as technetium-99m-pertechnetate ($^{99m}TcO_4$) has been used as a probes to understand the biological characteristic of the cancer cells by visualization, characterization and quantification of pathophysiological processes at the cellular and subcellular levels [4]. The interaction between cells and radiopharmaceutical, allows non-invasive detection and imaging of the cell growth and proliferation throughout the body which has long been recognised to be of significant value in the diagnosis and staging of cancer [5]. In this study, we determined the intercellular uptake of $^{99m}TcO_4$ by different types of cell lines and compare the uptake of different activity level and time of incubation. We also sought the correlation between the cell uptake and cell viability of the normal and cancerous type of cells.

MATERIALS AND METHODS

Materials.

All general chemical reagents and tissue culture reagents were purchased from Gibco, Life Technologies (USA) . The radionuclide ^{99m}Tc, was obtained from a molybdenum-99-technetium-99m (^{99}Mo-^{99m}Tc) generator located in the Nuclear Medicine, Oncology and Radiotherapy Department, School of Medicine, Universiti Sains Malaysia. The generator ELUMATIC III was purchased from the CIS Bio International (France). The Symbia-E gamma camera (Siemens Medical Solutions, Illinois, USA) was used to measure the count of $^{99m}TcO_4$ uptake by cells.

Cell culture and culture media.

The experiments were conducted using four types of cell lines: glial cells (SVG p12), glioma (DTBRG-05MG), HeLa and human fetal osteoblast cell (hFOB). Glial, glioma and hFOB cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) while HeLa cells were grown in Roswell Park Memorial Institute (RPMI) 1640 culture media with 10% Fetal Bovine Serum (FBS), 100 units/ml penicillin and 100 µg /ml streptomycin. All cells were incubated at 37°C and 5% CO_2 humidified atmosphere. The cells were grown in 75 ml flask until confluence and were harvested for experiments using trypsin-EDTA. The trypsinized cells were plated in 6 well plates and were incubated for 24 hours before the experiments.

Preparation of $^{99m}TcO_4$.

The $^{99m}TcO_4$ were prepared from $^{99}Mo/^{99m}Tc$ generator, ELUMATIC III which produced an elution of a clear and colourless solution of sodium pertechnetate. The volume of the eluted $^{99m}TcO_4$ solution was around 5 ml with radioactivity of 200, 400, 600, 800 and 1000 µCi. The activity was measured and verified using a dose calibrator.

Determination of $^{99m}TcO_4$ uptake into cell cultures.

The cells were incubated with $^{99m}TcO_4$ of different activities at 30 minutes, 1 hour and 1.5 hours of incubation time. After incubation, the culture media were removed and the radioactivity in the culture media were

counted using a dose calibrator. The cells were then washed three times with phosphate buffer saline (PBS) to remove the remaining $^{99m}TcO_4$ on the cell monolayers. Cells were detached from the culture plate by adding 0.5 ml of trypsin-EDTA and then cell were re-suspended in fresh media. The cell suspension were then centrifuge at 15,000 RPM for 5 minutes. The $^{99m}TcO_4$ uptake by the cells was measured using gamma camera and the result was expressed as the counts per minute (CPM). After the uptake measurement, the cell viability assay using trypan blue exclusion method were performed to determine the percentage of cell viability. The experiment was performed twice to confirm the reproducibility of the result.

RESULTS AND DISCUSSIONS

The data illustrated in figure 1 shows that, the maximum uptake by hFOB cell occurs at the 1.5 hours of incubation. There are no differences of uptake between 0.5 hours to the 1 hours. Highest percentage uptake were observed at the lowest activity of $^{99m}TcO_4$ and percentage uptake were decreasing with increasing activity. The data in the figure 2 shows the similar trend in $^{99m}TcO_4$ cellular uptake by glial and glioma with the hFOB cell line. The intercellular uptake for this both types of cells are relatively maximum at the lowest activity and decreased with increasing activity of $^{99m}TcO_4$. The glial recorded the higher percentage uptake at 9.44 $\pm$ 0.09 % than the glioma cell line with 7.44 $\pm$ 2.12% percentage uptake at 200µCi activity. However, the percentage uptake at higher activity show no significant differences. The figure 3 summarise the percentage uptake of all four cells lines at different activity of $^{99m}TcO_4$. The HeLa cell line shows the highest percentage uptake, 11.21 $\pm$ 0.69%, while the lowest uptake was observed at 7.44 $\pm$ 2.12% for glioma. This is followed by hFOB and glial with percentage uptake of 10.02 $\pm$ 1.41% and 9.42 $\pm$ 0.09%, respectively. Correlation between intercellular percentage uptake and cell viability are presented in figure 4. High cell viability increase the intercellular uptake of the $^{99m}TcO_4$ and when the viability is low, its lead to the low intercellular uptake of $^{99m}TcO_4$.

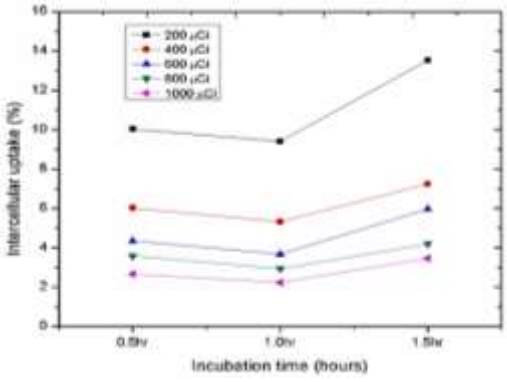

Figure 1: Intercellular percentage uptake at different $^{99m}TcO_4$ incubation time for hFOB cell line.

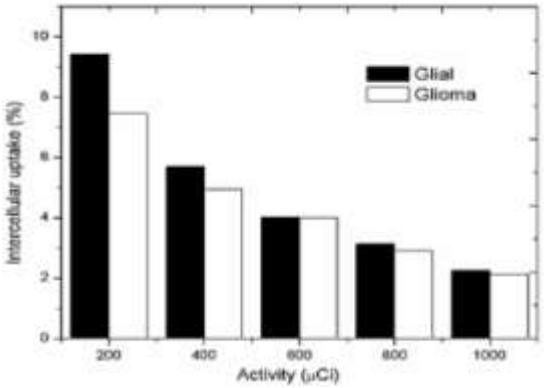

Figure 2: Intercellular percentage uptake of $^{99m}TcO_4$ by Glial and Glioma.

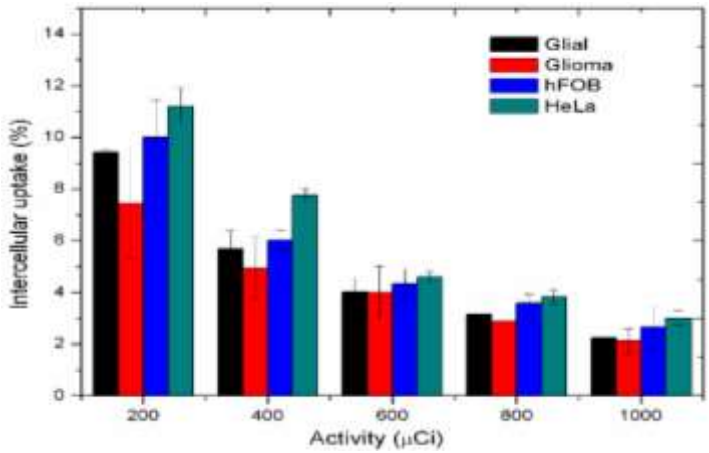

Figure 3: Intercellular uptake of $^{99m}TcO_4$ for different types of cell lines. The measurement were performed after 30 minutes incubation at 37°C and humidified with 5% CO_2.

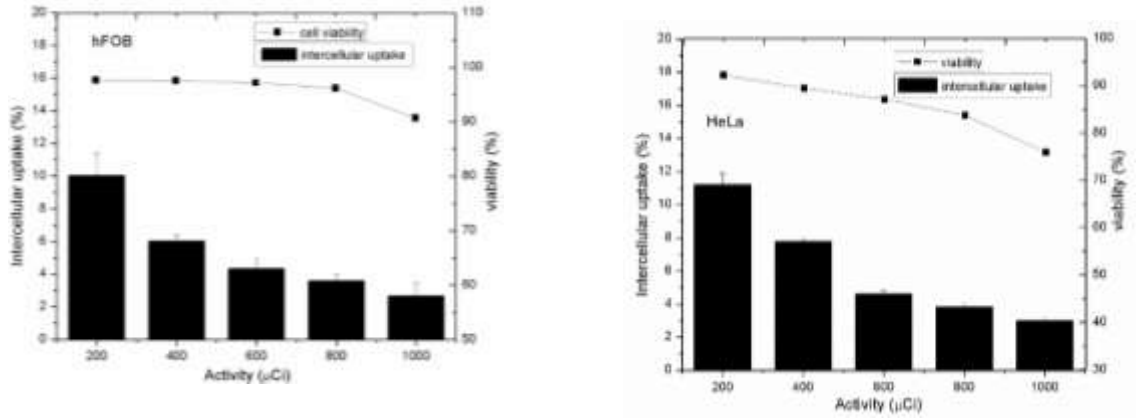

Figure 4: Intercellular hFOB and HeLa uptake in correlation with cell viability. The decreased viability of cell lines will affect the uptake percentage of both cancer and normal cell lines.

The results indicate that the intercellular uptake were maximum at lowest activity for all type of cells and longer incubation time have no significant effects. Optimal cell incubation time were found to be around 30 minutes and longer incubation time may affect the radiation counts as a results to the short half-life of $^{99m}TcO_4$. The intercellular uptake of $^{99m}TcO_4$ by different type of cells linked to the characteristic of the cells such as metabolic activity, cellular function and doubling time. Cancer cell such as HeLa is rapidly dividing type of cells were observed to have more uptake of $^{99m}TcO_4$ compare to slow dividing cell such as hFOB. However, comparison between normal and cancerous brain cells shows no significant difference in the uptake probably linked to other factor such as drug resistant characteristic and physiological parameters such as plasma membrane potential and intracellular pH [6, 7, 8].

CONCLUSION

The findings of this study suggest that the intercellular uptake of $^{99m}TcO_4$ is highly dependent on the type of cells despite no significant different of uptake was found between normal and cancer cell lines. The level of

radioactivity is also an important factor that influences the uptake of $^{99m}TcO_4$ into the cells. The results also shows correlation between the cellular uptake and the cell viability. Further studies need to be conducted to confirm the relationship between radiotracer uptake and the cellular characteristics of the cells.

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