Evaluation of Combined Contrast Agent using N-(p-maleimidophenyl) Isocyanate Linker-mediated Synthesis for Simultaneous PET-MRI

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ABSTRACT

In this paper, a combined ¹⁸F-FDG (fluorodeoxyglucose) and MNP (magnetic nanoparticles) contrast agent was synthesized using N-(p-maleimidophenyl) isocyanate as the crosslinker for use in simultaneous PET-MRI scans. PET-MRI images were acquired and evaluated before and after injection of the combined contrast imaging agent (¹⁸F-FDG labeled MNP) from a glioma stem cell mouse model. After setting the region of interest (ROI) on each acquired image, the area of the lesion was calculated by segmentation. As a result, the PET image was larger than the MRI. In particular, the simultaneous PET-MRI images showed accurate lesions along with the surrounding soft tissue. The mean and standard deviation values were higher in the MRI images alone than in the PET images or the simultaneous PET-MRI images, regardless of whether the contrast agent was injected. In addition, the simultaneous PET-MRI image values were higher than for the PET images. For PSNR experiments, the original image was PET Image using ¹⁸F-FDG, MRI using MNPs, and MRI without contrast medium, and the target image was simultaneous PET-MRI image using ¹⁸F-FDG labeled MNPs contrast medium. As a result, all of them appeared significantly, suggesting that the ¹⁸F-FDG labeled MNPs contrast medium is useful. Future research is needed to develop an agent that can simultaneously diagnose and treat through SPECT-MRI imaging research that can use various nuclides.

Keywords: Simultaneous PET-MRI, Imaging agents, Evaluation contrast effect, Image processing, PSNR, Magnetic resonance imaging

I. INTRODUCTION

Combining a single device to simultaneously acquire two data sets from Positron Emission Tomography(PET) and Magnetic Resonance Imaging(MRI) scans has been of interest in research and clinical applications since before the advent of combined Positron Emission Tomography-Computed Tomography(PET-CT) devices, which can be combined due to the complementary nature of the information provided by the two powerful imaging techniques. PET uses radionuclides that decay by positron emission and is used to analyze a wide range of biological processes with very high molecular sensitivity. However, PET lacks the spatial resolution to provide the anatomical detail needed for clinical interpretation. On the other hand, MRI provides high-resolution anatomical images with excellent soft tissue contrast, even if used in a typical pulse sequence. More advanced MRI sequences allow the study of water diffusion, blood oxygen concentration-
dependent contrast, and relative concentrations of various metabolites, and contrast can be further enhanced with the addition of gadolinium and iron oxide[3]. Although Positron Emission Tomography-Magnetic Resonance Imaging(PET-MRI) scan data can be fused, fusion methods can cause erroneous scan values because the scan values fluctuate with time. Simultaneous Positron Emission Tomography- Magnetic Resonance Imaging (Simultaneous PET-MRI) scans do not cause errors in data according to scan time, and spatial registration between two images, workflow, and comfort can be improved for patients who need to undergo both examinations[4,5]. In this paper, we examined images that were acquired using a combined contrast agent using the N-(p-maleimidophenyl) isocyanate (PMPI) crosslinker to synthesize the nuclide $^{18}$F-FDG (fluorodeoxyglucose) required for PET with the contrast agent required for MRI Magnetic Nanoparticles(MNPs) for use in simultaneous PET-MRI scans. Images before and after contrast agent injection were compared. All animal experiments were conducted with the approval of the Association for Assessment and Accreditation of Laboratory Animal Care(AAALAC) International.

II. MATERIAL AND METHODS

1. Simultaneous PET-MRI

Simultaneous PET-MRI uses radiopharmaceuticals that emit positrons and PET that can display biochemical and functional images of the human body in three dimensions and uses superconducting magnets and radio waves to protect tissues and blood vessels of the human body. It is a device that can simultaneously perform MRI, which examines humans in three dimensions[5]. Since it is an all-in-one device, PET-MRI simultaneously acquires both images such that examination time can be reduced by nearly half compared to the sequential equipment that obtains PET and MRI images sequentially[6]. The combination of PET and MRI is a significant technological advance in the measurement and processing of imaging signals alongside the influence of the magnetic field by the superconducting magnet of MRI, attenuation and motion compensation, and the design error of new RF coils in the amplification device of the PET ratio[7,8]. PET-MRI is a fusion imaging system that combines PET showing ultra-sensitive molecular imaging and MRI capable of high-resolution functional imaging[9]. Compared to PET-CT, PET-MRI exposes the patient to less radiation, as up to 70% of the dose received from PET-CT scans is due to Computed Tomography(CT), and demonstrates higher soft tissue contrast[9].

Fig. 1. Simultaneous Multiparametric PET/MRI.

2. PMPI linker-mediated PET-MRI contrast agent

Recently, medical imaging using hybrid technology has been widely accepted and used in clinical practice. Since simultaneous PET-MRI offers significant advantages over well-established PET-CT, including superior contrast and resolution and reduced ionizing radiation[11], simultaneous PET-MRI is useful in oncological imaging of areas such as the brain, head and neck, liver, and pelvis. Nanoscale particles can exhibit special physical and biological behaviors and unique interactions with biomolecules[12].

Nanoparticles have a large surface area and unique functions that alter pharmacokinetics, prolong vascular circulation time, improve extravasation capacity, ensure enhanced biodistribution in vivo, and induce sustained and controllable delivery[13]. In addition,
when a specific targeting ligand is conjugated to a nanoparticle, the nanoparticle can realize its target binding ability to a diseased region\textsuperscript{[14]}. Nanocarriers penetrate through microvessels with improved permeability and are then absorbed into cells, resulting in highly selective payload accumulation at the target site\textsuperscript{[15]}.

Over the past few decades, many traditional medical imaging techniques have been established for routine laboratory and clinical use. These imaging techniques, including optical imaging (OI), CT, MRI, ultrasound (US), and PET-single photon emission computed tomography (PET/SPECT) radionuclide imaging, have been widely applied in small-scale experiments and have shown excellent performance\textsuperscript{[16]}. Molecular imaging differs from conventional imaging in that it is used to image specific targets or pathways using probes known as biomarkers. Biomarkers must interact very specifically with their surrounding environment and change their image in response to molecular changes occurring within the region of interest (ROI). Molecular imaging agents are endogenous molecular or exogenous probes used to visualize, characterize, and quantify biological processes in living systems. Different imaging techniques in terms of sensitivity, resolution, and complexity often require specific contrast agents to achieve satisfactory contrast enhancement in visualization reconstructions\textsuperscript{[16]}.

Here, we describe a new PET-MRI combined contrast medium. Iron oxides (IONPs) are favored for T2 and T2*-weighted MRI imaging. There are several methods for the chemical synthesis of iron oxide nanoparticles. Among these methods, coprecipitation of Fe\textsuperscript{2+} and Fe\textsuperscript{3+} ions in a basic aqueous medium (NaOH or NH\textsubscript{4}OH solution) is the simplest, but generally polydisperse non-crystallized nanoparticles are obtained\textsuperscript{[17]}. To avoid these disadvantages, iron oxide nanoparticles that are monodisperse and of uniform crystallinity were prepared using a thermal decomposition method. Then, the hydrophobic iron oxide nanoparticles can be coated with phospholipids, silica, or an amphiphilic polymer as a shell to exhibit excellent solubility and biocompatibility in vivo\textsuperscript{[17]}. In the simultaneous PET-MRI units, the PET is placed within the MR bore. To acquire simultaneous PET-MRI images, a combined imaging agent must be metabolized simultaneously in the body\textsuperscript{[18]}. Therefore, the MRI and PET contrast agents should be synthesized as one agent. Here, we used PMPI to crosslink MNPs for MRI imaging with \(^{18}\)F-FDG for PET imaging as a single contrast agent for combined PET-MRI imaging (Fig. 2).

In general, the MNPs are coated with oleic acid to maintain uniform dispersion. However, the coated MNPs cannot be directly injected in vivo due to toxicity. Therefore, after removing the toxicity with hexane, which is usually a strong acid, it is substituted with a biocompatible polymer biomaterial\textsuperscript{[19]}. In this study, \(^{18}\)F-FDG was attached to the MNPs by electrostatic attraction to the MNP surface by removing oleic acid using specific conditions and leaving an SH-group that is frequently expressed on the metal surface\textsuperscript{[20]}. The \(^{18}\)F-FDG and MNP attachment occurs when they are subjected to a physical reaction by centrifugation, whereby SH-MNPs with a relatively heavy molecular weight, exist on the inside by centrifugal force and the water-soluble \(^{18}\)F-FDG with a relatively lighter molecular weight, stays on the surface\textsuperscript{[21]}. The magnetic SH- group and the OH-group of \(^{18}\)F-FDG are conjugated by the PMPI crosslinker.
3. Image evaluation

Image quality evaluation characterizes the content and texture of an image. Basically, evaluation metrics can be categorized into primary, secondary, and higher order scales. Primary metrics focus on properties such as the mean intensity, standard deviation, and variance and first-order metrics only have an effect on individual pixels in the image. First-order metrics do not account for spatial relationships between pixels, and therefore do not address neighbor relationships\[22,23\]. On the other hand, quadratic or higher metrics measure the properties of two or more pixels that occur relative to each other at a specific location. In medical images, the mean is often used as a matrix representative value of pixels\[24\]. The standard deviation, seen in scatter plots, is a representative number indicating how spread out the medical image pixel data is around the mean\[24\]. A standard deviation close to 0 means that the data values are concentrated near the mean, and a larger standard deviation means that the data values are spread out\[25\].

Peak signal to noise ratio (PSNR) is the maximum signal-to-noise ratio. It is an objective measurement method that numerically indicates the difference between the image before and after contrast medium injection during medical image evaluation. PSNR is most easily defined via the mean squared error (MSE)\[25,26\]. For a before contrast agent injection m×n monochrome image I, and after contrast agent injection image approximation K, MSE is defined as:

\[
MSE = \frac{1}{mn} \sum_{i=0}^{m-1} \sum_{j=0}^{n-1} [I(i,j) - K(i,j)]^2
\]  

The PSNR (in dB) is defined as:

\[
PSNR = 10 \cdot \log_{10} \left( \frac{MAX^2}{MSE} \right) = 20 \cdot \log_{10} \left( \frac{MAX}{\sqrt{MSE}} \right) = 20 \cdot \log_{10}(MAX) - 10 \cdot \log_{10}(MSE)
\]  

Here, MAX is the maximum possible pixel value of the image. When the pixels are represented using an 8 bit per sample, this is 255. More generally, when samples are represented using linear pulse-code modulation (PCM) with B bits per sample, MAX is 2^B-1. Image processing for image evaluation was performed by writing an M-program using the MATLAB® Image Processing Toolbox as shown in Fig. 3.

![Fig. 3. Example of M-programming for image evaluation.](image)

III. EXPERIMENT AND RESULT

The experiment was carried out as outlined in Fig. 4. After preparing the ¹⁸F-FDG labeled MNPs combined contrast agent for simultaneous PET-MRI, images were acquired with a simultaneous PET-MRI device. The acquired and stored images were pre-processed with an 8-bit depth of 256 x 256 pixels for image processing, and then entered into the MATLAB® program. First, the following images were processed: pre-injection MRI images, MNP contrast agent MRI images, ¹⁸F-FDG contrast agent images.
PET images, and $^{18}$F-FDG labeled MNP contrast agent simultaneous PET-MRI images. Then, the images were used to define the ROI, followed by segmentation of the ROI. Next, the acquired images were expressed as a three-dimensional figure with a surface plot. Finally, PSNR values were obtained to evaluate the efficiency of the combined $^{18}$F-FDG labeled MNP PET-MRI contrast agent. Finally, simultaneous PET-MRI images were acquired after injection of the $^{18}$F-FDG labeled MNP contrast agent. Table 1 shows representative acquired images.

2. Processing acquired images

After preprocessing the obtained experimental images to 256 X 256 pixels and 8 bits in-depth, the mean value and standard deviation were calculated. Table 2 shows the mean value and standard deviation for each set of experimental images.

![Flow-chart of the experimental process](image)

**Fig. 4. Flow-chart of the experimental process.**

1. Image acquisition by the simultaneous PET-MRI

For the experimental images, a coronal section was obtained four weeks after transplanting U87 glioma stem cells into a mouse model. MRI images were acquired with no contrast agent and after MNP contrast agent injection. PET images were acquired after the injection of $^{18}$F-FDG contrast agent, and finally, simultaneous PET-MRI images were acquired after injection of the $^{18}$F-FDG labeled MNP contrast agent. Table 1 shows representative acquired images.

The ROI was set for the following acquired images: simultaneous PET-MRI ($^{18}$F-FDG labeled MNP contrast agent), PET ($^{18}$F-FDG), MRI (MNP), and MRI (no contrast agent) using the MATLAB M-program. Then, the set ROI was segmented, and a surface plot was performed. The performance results are shown in Tables 3 through 6.

**Table 2. Mean value and standard deviation of experimental images**

<table>
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<tr>
<th>Device</th>
<th>Imaging agent</th>
<th>Method</th>
<th>Value</th>
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<tbody>
<tr>
<td>Simultaneous</td>
<td>$^{18}$F-FDG labeled MNP</td>
<td>Mean</td>
<td>32.7622</td>
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<tr>
<td>PET-MRI</td>
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<td>Standard Deviation</td>
<td>38.8094</td>
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<tr>
<td>PET</td>
<td>$^{18}$F-FDG</td>
<td>Mean</td>
<td>16.2275</td>
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<tr>
<td>MRI</td>
<td>MNPs</td>
<td>Standard Deviation</td>
<td>23.1136</td>
</tr>
<tr>
<td>MRI</td>
<td>None</td>
<td>Mean</td>
<td>59.0839</td>
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<tr>
<td>MRI</td>
<td>MNP injection</td>
<td>Standard Deviation</td>
<td>60.5827</td>
</tr>
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</table>

**Table 1. Image acquisition via the simultaneous PET-MRI device**

<table>
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<tr>
<th>MRI</th>
<th>PET</th>
<th>Simultaneous PET-MRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>$^{18}$F-FDG injection</td>
<td>$^{18}$F-FDG labeled MNP injection</td>
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</table>

![Images](image)
Table 3. Simultaneous PET-MRI ($^{18}$F-FDG labeled MNP contrast agent) image processing

<table>
<thead>
<tr>
<th>$^{18}$F-FDG labeled MNPs</th>
<th>ROI</th>
<th>Segmentation</th>
<th>Surface Plot</th>
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Table 4. PET ($^{18}$F-FDG contrast agent) image processing

<table>
<thead>
<tr>
<th>$^{18}$F-FDG Injection</th>
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<th>Segmentation</th>
<th>Surface Plot</th>
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<td><img src="image7.png" alt="Image" /></td>
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Table 5. MRI (MNP contrast agent) image processing

<table>
<thead>
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<th>MRI MNP injection</th>
<th>ROI</th>
<th>Segmentation</th>
<th>Surface Plot</th>
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<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
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Table 6. MRI (no contrast agent) image processing

<table>
<thead>
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<th>MRI Pre-injection</th>
<th>ROI</th>
<th>Segmentation</th>
<th>Surface Plot</th>
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<td><img src="image15.png" alt="Image" /></td>
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</table>
Table 7. Results of PSNR between PET (\(^{18}\)F-FDG) and PET-MRI (\(^{18}\)F-FDG labeled MNP)

<table>
<thead>
<tr>
<th>Origin image</th>
<th>Target image</th>
<th>Method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET ((^{18})F-FDG)</td>
<td>Simultaneous PET-MRI ((^{18})F-FDG labeled MNP)</td>
<td>MSE</td>
<td>1690.1609</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RMSE</td>
<td>41.1116</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSNR</td>
<td>15.8515 dB</td>
</tr>
</tbody>
</table>

Table 8. Results of PSNR between MRI (MNP) and PET-MRI (\(^{18}\)F-FDG labeled MNP)

<table>
<thead>
<tr>
<th>Origin image</th>
<th>Target image</th>
<th>Method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI (MNP)</td>
<td>Simultaneous PET-MRI ((^{18})F-FDG labeled MNP)</td>
<td>MSE</td>
<td>1871.9511</td>
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<tr>
<td></td>
<td></td>
<td>RMSE</td>
<td>43.2661</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSNR</td>
<td>15.4079 dB</td>
</tr>
</tbody>
</table>

Table 9. Results of PSNR between MRI (no contrast agent) and PET-MRI (\(^{18}\)F-FDG labeled MNP)

<table>
<thead>
<tr>
<th>Origin image</th>
<th>Target image</th>
<th>Method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI (none)</td>
<td>Simultaneous PET-MRI ((^{18})F-FDG labeled MNP)</td>
<td>MSE</td>
<td>3254.2386</td>
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<tr>
<td></td>
<td></td>
<td>RMSE</td>
<td>57.0459</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSNR</td>
<td>13.0063 dB</td>
</tr>
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</table>

**IV. DISCUSSION**

The obtained experimental images were segmented after setting the ROI and the area of the lesion was examined by attaching a grid to the segmented image. As a result, it was found that there was no significant difference in the lesion area in the MRI image regardless of the use of MNP as the contrast agent. Simultaneous PET-MRI images using the \(^{18}\)F-FDG labeled MNP contrast agent and PET images using the \(^{18}\)F-FDG contrast agent showed clearer outlines of the lesion compared to MRI alone\(^{[27]}\). Table 10 shows the mutual comparison of the area of the lesion by the grid, and shows that PET has better accuracy than MRI. It can be seen that the images acquired from PET show a clear distinction between surrounding tissues and lesions relative to MRI\(^{[27,28]}\). In PET and simultaneous PET-MRI, the PET image shows a large lesion area, which is thought to be due to the difference between the used contrast imaging agents (\(^{18}\)F-FDG and \(^{18}\)F-FDG labeled MNP)\(^{[29]}\).

Simultaneous PET-MRI imaging, similar to MRI, is capable of observing high-contrast soft tissue, which has the advantages of MRI\(^{[30]}\).

Fig. 5 shows the average value and standard deviation of the signal for each experimental image. The mean and standard deviation values were higher for the MRI images than both the PET or simultaneous PET-MRI images, regardless of whether contrast agents were used, and simultaneous PET-MRI images were higher than PET images. These results indicate that MRI is an imaging device with a high signal-to-noise ratio(SNR). The reason why simultaneous PET-MRI images have higher mean and standard deviation values than PET images is thought to be due to the action of MNP.
Fig. 5. Mean value and standard deviation for each pixel of experimental images.

Fig. 6 shows the PSNR as calculated below:

1) PSNR was calculated using simultaneous PET-MRI images (\(^{18}\text{F-FDG}\) labeled MNP) as the target image and PET images (\(^{18}\text{F-FDG}\)) as the original image.

2) PSNR was calculated using simultaneous PET-MRI images (\(^{18}\text{F-FDG}\) labeled MNP) as the target image and MRI images (MNP) as the original image.

3) PSNR was calculated using simultaneous PET-MRI images (\(^{18}\text{F-FDG}\) labeled MNP) as the target image and MRI images (no contrast agent) as the original image.

As shown in Fig. 6, the PSNR obtained using the simultaneous PET-MRI image (\(^{18}\text{F-FDG}\) labeled MNP) as the target image and the PET image (\(^{18}\text{F-FDG}\)) as the original image had the highest value of 15.85 dB.

V. CONCLUSION

In this paper, the enhancement effect of the \(^{18}\text{F-FDG}\) labeled MNP simultaneous PET-MRI scan imaging contrast agent was evaluated in a glioma stem cell mouse model. For evaluation, images were acquired with the simultaneous PET-MRI \(^{18}\text{F-FDG}\) labeled MNP contrast agent, PET images alone with \(^{18}\text{F-FDG}\) contrast agent, MRI images alone with MNP contrast agent, and MRI images with no contrast agent were obtained for comparison.

After setting the ROI on each acquired image, the area of the lesion was calculated by segmentation. As a result, the PET image was larger and more accurate than the MRI image. In particular, the simultaneous PET-MRI image showed accurate lesions and the surrounding soft tissue. The mean and standard deviation values were higher for the MRI images than both the PET or simultaneous PET-MRI images, regardless of whether contrast agents were used, and simultaneous PET-MRI images were higher than PET images. The PSNR value showed a significant value in all experiments and showed the greatest value when the simultaneous PET-MRI image (\(^{18}\text{F-FDG}\) labeled MNP) was used as the target image and the PET image (\(^{18}\text{F-FDG}\)) was used as the original image.

In conclusion, the usefulness of the \(^{18}\text{F-FDG}\) labeled MNP combined contrast agent as a simultaneous PET-MRI imaging agent was confirmed. Future research is required to develop an agent that can simultaneously diagnose and treat through SPECT-MRI imaging that can be used with various nuclides.

Reference


Evaluation of Combined Contrast Agent using N-(p-maleimidophenyl) Isocyanate Linker-mediated Synthesis for Simultaneous PET-MRI


동시 PET-MRI를 위한 N-((p-maleimidophenyl) isocyanate linker-매개 합성을 이용한 복합 조영제의 평가

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3연세대학교세브란스병원-한국생명공학연구소 병설 의료융합연구소
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요 약
이 논문에서는 결합된 PET(fluorodeoxyglucose, 18F-FDG)와 MRI(magnetic nanoparticles, MNP) 조영제를 동시 PET-MRI 스캔에 사용하기 위한 가교제로 N-(p-maleimidophenyl) isocyanate를 사용하여 합성하는 방안을 제안하였다. 실험은 신경교종 줄기 세포 마우스 모델에서 결합 조영제(18F-FDG로 표지된 MNP)를 주입하기 전후에 PET-MRI 이미지를 획득하고 평가하였다. 획득한 각 영상에 대해 관심영역(ROI)을 설정한 후, 분할하여 병변의 면적을 계산하였을 때 PET 영상이 MRI 영상보다 더 크고 정확했다. 특히 동시 PET-MRI 영상은 주변 연조직과 함께 정확한 병변을 묘사하였다. 평균 및 표준편차 값은 조영제 주입 여부에 관계없이 PET 영상 또는 PET-MRI 동시 영상보다 MNP 단독 영상에서 더 높게 나타났다. 또한 동시 PET-MRI 영상 값이 PET 영상보다 평균 및 표준편차 값이 높게 나타났다. 18F-FDG 라벨링된 MNP 조영제와 동시 PET-MRI 영상을 표적 영상으로 사용하고 18F- FDG 조영제만을 원본 이미지로 사용했을 때의 피크 신호 대 잡음비(PSNR) 값을 모든 실험에서 유의미하게 나타났다. 결론적으로 동시 PET-MRI 영상에서 결합된 18F-FDG 표지 MNP 조영제가 유용함을 확인하였다. 다양한 핵종을 사용할 수 있는 SPECT-MRI 영상 연구를 통해 진단과 치료를 동시에 할 수 있는 체계를 개발하기 위해서는 향후 연구가 필요할 것이다.

중심단어: 동시PET-MRI, 조영제, 조영효과 평가, 영상처리, 최대신호대잡음비(PSNR)

연구자 정보 이력

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