Pixelized Measurement of ^{99m}Tc -HDP Micro Particles Formed in Gamma Correction Phantom Pinhole Scan: a Reference Study

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Abstract

Purpose Currently, traumatic bone diseases are diagnosed by assessing the micro ^{99m}Tc -hydroxymethylene diphosphonate (HDP) uptake in injured trabeculae with ongoing osteoneogenesis demonstrated by gamma correction pinhole scan (GCPS). However, the mathematic size quantification of micro-uptake is not yet available. We designed and performed this phantom-based study to set up an in-vitro model of the mathematical calculation of micro-uptake by the pixelized measurement.

Methods The micro ^{99m}Tc -HDP deposits used in this study were spontaneously formed both in a large standard flood and small house-made dish phantoms. The processing was as follows: first, phantoms were flooded with distilled water and ^{99m}Tc -HDP was therein injected to induce micro ^{99m}Tc -HDP deposition; second, the deposits were scanned using parallel-hole and pinhole collimator to generally survey ^{99m}Tc -HDP deposition pattern; and third, the scans underwent

gamma correction (GC) to discern individual deposits for size measurement.

Results In original naïve scans, tracer distribution was simply nebulous in appearance and, hence, could not be measured. Impressively, however, GCPS could discern individual micro deposits so that they were calculated by pixelized measurement. Phantoms naturally formed micro ^{99m}Tc -HDP deposits that are analogous to ^{99m}Tc -HDP uptake on in-vivo bone scan. The smallest one we measured was 0.414 mm. Flooded phantoms and therein injected ^{99m}Tc -HDP form nebulous micro ^{99m}Tc -HDP deposits that are rendered discernible by GCPB and precisely calculable using pixelized measurement.

Conclusions This method can be used for precise quantitative and qualitative diagnosis of bone and joint diseases at the trabecular level.

Keywords Gamma correction · Micro deposit · ^{99m}Tc -HDP · Pinhole scan · Pixelized measurement

Introduction

^{99m}Tc -hydroxymethylene diphosphonate (HDP) bone scan, a molecular imaging, has been long and widely used in daily practice for the macroscopic anatomical diagnosis of bone and joint diseases. With the recent development of gamma correction pinhole scan (GCPS), many common bone diseases are now diagnosed at the dimension of trabeculae. They include occult fracture [1], trabecular microfracture [2], and stress fracture, infection, and tumor [3]. To our knowledge, however, a mathematical method for the size quantification of such microscopic ^{99m}Tc -HDP uptake has not been published.

Because of the impracticability to obtain useful bone materials directly from patients, we designed and performed this study using phantoms. The aim was to set up an in-vitro model

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of the size measurement of microscopic ^{99m}Tc -HDP uptake that can be used clinically in in-vivo ^{99m}Tc -HDP bone scan. Thus, the objectives of this study were first to secure the reliable source of microscopic ^{99m}Tc -HDP deposits for micrometric measurement using standard flood phantom and handy small dish phantom; second, to demonstrate individual deposits with high ^{99m}Tc -HDP radioactivity by GCPS [1, 2]; and third, to mathematically calculate their size by pixelized measurement [4, 5]. Microscopic ^{99m}Tc -HDP deposits were easily formed in both large standard and small house-made dish phantoms, readily processed by GCPS and precisely calculated.

Gamma correction (GC) is a widely used image processing algorithm that can transform an image pixel value nonlinearly to control the gray or color scale of an image on a computer monitor to adjust the pictorial contents for specific purpose [6]. Impressively, GC keeps high ^{99m}Tc -HDP uptake unwashed, for example, in the fracture with actively forming callus, whereas it cleanly washes out and erases low uptake in bone edema and hemorrhage [1, 3]. GC can be performed in any desk computer. On the other hand, the pixelized measurement, a recently developed quantitative calculation, can be used for the analysis of computed images [4, 5]. We describe and discuss the methods and results.

Materials and Methods

Formation and Measurement of Microscopic ^{99m}Tc -HDP Deposits

The microscopic ^{99m}Tc -HDP deposits used in this study formed naturally in the water-flooded phantom into which ^{99m}Tc -HDP was injected (Fig. 1a1). Such deposit formation was found accidentally by the senior author. The acrylic planar flood phantom used was Model 043-054 of Biodex Medical Systems Inc., Shirley, NY. The dimension was $52 \times 71.1 \times 3.2$ cm. To begin with, the phantom was filled with 3000 ml of distilled water and 370 MBq (10 mCi) of ^{99m}Tc -HDP were therein injected through an injection port [7]. Next, the phantom was gently shaken manually for 20 min to diffuse and homogenize ^{99m}Tc -HDP in flood water. Despite shaking, however, ^{99m}Tc -HDP deposits formed along the tracer injection trail (Figs. 1a1 and 2). Those deposits, too small to see with unaided eyes, were magnified using 4-mm pinhole scanning for accurate measurement. Two hundred Kcounts were accumulated in 10 min (Fig. 1b1).

Pinhole Scanning

Pinhole scan was performed using a standard bar phantom (Model 243-800 $45.7 \times 45.7 \times 1.0$ cm, Biodex Medical Systems, Shirley, New York) paired with a planar flood

phantom 2.5 hr after intravenous injection of 370 MBq (10 mCi) of ^{99m}Tc -HDP using a 4-mm aperture pinhole collimator (Fig. 1b1; right column). Aperture-to-object distance (AOD) was 10 cm. Photon acquisition was 134 Kcounts and scan time was 10 min. The gamma camera used was E.CAM Signature Series digital scanner (Siemens, Erlangen, Germany). The reason for using bar phantom was that the scan image resolution of microscopic deposit is improved due to that the halation effect of neighboring deposits with high radioactivity is eliminated (Fig. 1b1 and b2).

Gamma Correction

Gamma correction was performed to distinctly visualize individual ^{99m}Tc -HDP deposits with unwashed high tracer uptake using a Photo Correction Wizard program (Fig. 1b2) [1]. Gamma value was raised to 95 to wash out low and intermediate radioactivity preserving high radioactivity unwashed. The correction was done in the following order: Exposure and auto-exposure to maximize uptake intensity, and done and save with a new name. Then, exposure and image-brightness control by raising gamma value to wash out noise uptake, and done and save the finished image with another new name. Starting from 50 (the default value), gamma value was usually raised to 95. The use of original naïve digital information and communications in medicine (DICOM) scan was an absolute mandate.

Reproducibility of ^{99m}Tc -HDP Deposit Formation in Dish and Flood Phantoms

The reproducibility of deposit formation was checked in two different ways using five small polyethylene dish phantoms and one large standard acrylic flood phantom. First, the pinhole scans of five dish phantoms were taken in one setting under the conditions of pinhole-target distance 10 cm and the field of view 14×14 cm² (Fig. 2a; left column). Each scan was processed using gamma correction (Fig. 2a; right column). One-hundred ml of distilled water was filled in each dish and 92.5 MBq (2.5 mCi) of ^{99m}Tc -HDP solution was therein added, and 420 Kcounts were accumulated in 2 min. The dimension of dish phantom was 10 cm in diameter and 3 cm in depth. The size of five small ^{99m}Tc -HDP deposits was calculated in each dish phantom using pixelized measurement (Fig. 2b1-b5). Second, the GCPS of ^{99m}Tc -HDP deposits was taken in standard flood phantom after the injection of 370 MBq (10 mCi) at 4.5-month interval, one on 05.27.2014 (spring) and the other on 10.12.2014 (autumn). The size of ten small deposits in each test was calculated using pixelized measurement (Fig. 3a and b).

Fig. 1 The formation and size calculation of micro ^{99m}Tc -HDP deposits in flood phantoms. **a1** Naïve parallel-hole planar scan of standard flood phantom shows un-measurable nebulous high ^{99m}Tc -HDP radioactivity along injection trail (red arrows; IP = injection port). Inset shows setup of planar scan of flood phantom (FP). **a2** Gamma correction planar scan shows pinpoint deposits which are too small to observe with naked eyes. **b1** Naïve pinhole scan of bar flood phantom shows un-measurable nebulous radioactivity. Inset shows setup of pinhole scan. PC, BP, and

PF denote pinhole collimator, bar phantom, and flood phantom, respectively. **b2** GCPS clearly discerns ^{99m}Tc -HDP deposits so that their size can be calculated using pixelized measurement. An exemplary deposit measures 0.435 mm ($\square$). **c1-3** Pixelized measurement. **c1** Smaller vertical or horizontal profile is extracted from ROI. **c2** Enlarged ROI in profile. Two points are picked to calculate FWHM. **c3** FWHM is obtained from line spread function by piecewise-polynomial interpolation

Validation of Absence of ^{99m}Tc -HDP Aggregation Using Chromatographic Analysis

For the assessment of the chromatographic characteristics of the deposits, the purity of ^{99m}Tc -HDP was checked using thin layer chromatography (ITLC-SG; Agilent Technologies; Lake Forest City, CA) and Whatman No. 1 Paper (Sigma-Aldrich, Gyeonggi-do, Korea) and radio-chromatograms were taken using a AR2000 TLC scanner (Bioscan, Washington DC) in two other additional dish phantoms. Each of two dishes was flooded with 5 ml of distilled water and 370 MBq/1.0 ml of ^{99m}Tc -HDP was therein added. One dish was kept still and the other one shaken to homogenize ^{99m}Tc -HDP. Two hours afterward, a small drop taken from the unshaken and shaken dish was subjected to TLC test.

Pixelized Measurement of the Size of Particulate ^{99m}Tc -HDP Deposits in GCPS

^{99m}Tc -HDP deposit size was calculated using pixelized measurement (Fig. 1c1-3) [4] in GCPSs of a bar phantom and five dish phantoms. FOV was $14 \times 14 \text{ cm}^2$ and matrix size was 512×512 . For calculation, the matrix of all pinhole scan images

was implemented as 256×256 by the image processing software. In GCPS, each microscopic spot was appointed as a region of interest (ROI). The image profile is presented in a line spread function denoting the signal intensity of the applied line. The Y-axis of image profile is signal intensity and the X-axis shows the pixel number in profile. In order to measure FWHM easily, the matrix size was then expanded to 512×512 as stated above. Because of the expansion of the number of pixel, the signal intensity value was assigned by the interpolation at the expanded bin which does not have the signal intensity value. To interpolate the between bins, the Gaussian curve fitting method was used by the in-house MATLAB code (Mathworks, R2011a, USA) [8]. The equation of the Gaussian curve fitting is as below.

$$y(x) = \frac{1}{\sigma\sqrt{2\pi}} \exp \left[-\frac{(x-x_0)^2}{2\sigma^2} \right]$$

y interpolated value
x original frame value
 x_0 mean value of the frame value
 σ standard deviation

Fig. 2 **a** Setup of dish phantom pinhole scanning (left column) and pre and post gamma correction images (right column). **#1—#5** High reproducibility of ^{99m}Tc -HDP deposits in five dish phantoms. Inset is

setup of pinhole collimator (PC) and dish phantom (DP). Five small deposits measured in each GCPS. Each GCPS shows ^{99m}Tc -HDP deposits measuring from 0.939 to 1.201 mm in size

From two different GCPSs of bar-and-flood-phantom tests, the sizes of ten small ^{99m}Tc -HDP particles was calculated for comparative evaluation (Fig. 3).

Statistics

The statistical analysis was performed using Medcalc software (version 10.1.7.0, Ostend, Belgi). Two-tailed

P-value less than 0.05 was regarded as statistical significance. Kruskal-Wallis test was used to compare pixelized measured sizes of five small deposits among the five dish phantoms. Mann-Whitney U test was used to compare pixelized measurement of ten small deposit sizes between the bar phantoms. Mann-Whitney U was also used for comparison of pixelized measurement of sizes of small deposits between bar and dish phantoms.

Fig. 3 The reproducibility of micro ^{99m}Tc -HDP deposits in standard flood source on scans performed at a 4.5-month interval. **a** First test shows the deposit to measure from 0.414 to 1.218 mm

(mean = 0.752 mm). **b** Second test shows the deposit to measure from 0.553 to 1.046 mm (mean = 0.692 mm). The smallest deposits measured were 0.414 mm and 0.563 mm in the first and second test, respectively

Fig. 4 Statistics. **a** The sizes of the five smallest deposits from five dish phantoms are not significantly different ($P = 0.331$, Kruskal-Wallis test). **b** There was also no significant difference of pixelized measured size of the ten smallest deposits between bar phantoms ($P = 0.427$, Mann-

Whitney test). **c** However, the sizes of deposits in five dish phantoms is significantly larger than that of deposits in flood source in bar phantoms ($P < 0.001$, Mann-Whitney test)

Results

The sizes of five small ^{99m}Tc -HDP deposits calculated in five dish phantoms ranged from 0.939 to 1.150 mm (mean = 1.0057 ± 0.1014) (Fig. 2) and the differences among the results of those five phantoms were statistically not significant, ($P = 0.331$; Fig. 4a). The sizes of ten small ^{99m}Tc -HDP deposits calculated in the first standard flood phantom test performed on 05.27.2014 and the second test performed on 10.12.2014 ranged from 0.414 to 1.218 mm (mean = 0.7520

± 0.2280) and from 0.553 to 1.046 mm (mean = 0.6915 ± 0.1663), respectively (Fig. 3a, b). The difference between the results was statistically not significant either ($P = 0.427$; Fig. 4b). However, the difference between the sizes of the deposits formed in standard flood phantom and dish phantoms was statistically significant ($P < 0.001$; Fig. 4c).

The R_f values of ^{99m}Tc -HDP, free ^{99m}Tc and ^{99m}Tc colloid are known to be 0.0, 1.0, and 0.0, respectively, at ITLC-SG/acetone condition, while 1.0, 1.0, and 0.0 at paper/saline condition [7]. In our TLC test (Fig. 5), the R_f values of the

Fig. 5 ^{99m}Tc -HDP solution used in this experiment contains no colloid or precipitate at the starting time-point, and there are no significant changes in TLC at any condition up to 2 hours. R_f values of ^{99m}Tc -HDP are 0.0 at ITLC-SG/acetone condition and 1.0 at paper/saline condition

prepared ^{99m}Tc -HDP were 0.0 at ITLC-SG/acetone condition and 1.0 at paper/saline condition denoting that prepared ^{99m}Tc -HDP solution did not contain colloid or precipitate at the starting time-point. Up to 2 hours after transferring the ^{99m}Tc -HDP solution to the polyethylene dish phantom, no significant changes were noted in the TLC.

Discussion

The naïve unprocessed microscopic ^{99m}Tc -HDP deposits formed in the flooded phantoms were typically dispersed and diluted presenting as nebulae and, hence, they were not identifiable as such on both planar parallel-hole (Fig. 1a1) and pinhole scans (Fig. 1b1). Of course, they were not measurable by any means. Impressively, GC can drastically change the imaging condition clearly bringing out microscopic deposits of nebulae on both scans (Fig. 1a2, b2). It was the result of that the ^{99m}Tc -HDP deposit with high radioactivity was not washed out, whereas the deposit with low or intermediate radioactivity was washed out. Unfortunately, however, the visualized deposits shown on parallel-hole scan were neither sharp nor large enough for precise calculation needing magnification that was accomplished by pinhole scan. Being magnified, microscopic deposits then became easily calculated using pixelized measurement.

For pixelized measurement, the line spread function was employed [4]. The function is a kind of image profile that reveals the intensity of signal from image matrix [5]. Actually, the size of ^{99m}Tc -HDP deposits shown in GCPS was calculated by measuring the FWHM of the signal peak (Fig. 1c1-3). Such mathematical calculation of FWHM value from image profile is deservedly true solving the problem of penumbra in visual measurement. It is to be emphasized that pinhole scan cannot distinguish the deposits with high radioactivity from those with low radioactivity unless treated using GC that washes out and eliminates the noises of low radioactivity [1] (Fig. 1b1). Thus, GCPS could discern ^{99m}Tc -HDP deposits so that their size was easily measured. The smallest deposit we measured in this study was 0.414 mm. The reproducibility of particulate ^{99m}Tc -HDP deposit formation and measurement was excellent in both standard flood phantom and dish phantom.

In order to characterize the chemical nature of deposits, we performed radio-TLC test and the results indicated that they are neither colloid nor precipitate. It was shown that deposits naturally form in plastic phantom through some unknown causative processes that require further investigation. In passing, it was tempting to hypothesize that an interaction between ^{99m}Tc -HDP and polyethylene of dish phantom might have caused deposits the size of which was larger than those formed in water phantom which was made of acrylic plastic. Our surgical microscopic scrutiny revealed no detectable physical alteration in the surface of polyethylene or acrylic phantom.

Conclusions

The results of our study indicate that water-flooded standard flood phantom and house-made dish phantom and therein injected ^{99m}Tc -HDP naturally form in-vitro microscopic deposits that are analogous to the in-vivo microscopic uptake on clinical ^{99m}Tc -HDP bone scan. Their size can be precisely calculated by pixelized measurement. This method can be used as an easy and economical means of the size measurement of the microscopic ^{99m}Tc -HDP uptake in bone scan. The smallest microscopic deposit we measured in this study is 0.414 mm.

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Conflict of Interest Joo-Young Jung, Gi Jeong Cheon, Yun-Sang Lee, Seunggyun Ha, Mi-Hye Chae, Yong-An Chung, Do Kyun Yoon, and Yong-Whee Bahk declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human participants or animals performed by any of the authors.

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