In Vivo Monitoring the Circulation by Hard X-ray Microscope and Cell Images Obtained by High-Resolution Zone Plate X-ray Microscope

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INTRODUCTION

Nanoparticles were found wild applications in medicine; the most highlighted was its application as drug delivery carriers. The surface of nanoparticels can be modified or conjugated with specific antibodies or fluorescence dyes to identify proteins or label the tumor cells. Most of the imaging techniques, such as MicroCT (computed tomography) or PET (positron emission tomography), can only measure the distribution of nanoparticles in animal certain amount of time after injection. The temporal and spatial resolutions were greatly compromised and insufficient to trace the circulation and deposition of nanoparticles in real time. The Synchrotron Radiation (SR) based microradiology we developed offers very higher temporal and spatial resolution (<1 ms and <1 μm) and we demonstrate in this work that it is ideal to study *in vivo* the circulation and accumulation of the gold nanoparticles.

The mouse without any contrast agent showed clear bone structure and thoracic cavity (Figure 1. a). Bone showed darker contrast than the soft tissue. The distribution of 600μl lipodol® contrast agent in the vessels of the organs, this solution could not diluted by the body fluid and could easily presented clear vessels structure in the mouse body (Figure 1. b). On the contrary, telebrix® contrast agent was easily diluted by the body. All the organs showed darken contrast and without clear fringe, except bladder (Figure 1. c).

The lipodol® contrast agent showed clear vessel structure of heart, liver and kidney. The most impress was the resolution of the image can get the vessel size down to about 20 μm. The image principle of x-ray microscopy just like the optical microscope, for the reason the resolution should reach to 0.2μm.The resolution of the synchrotron x-ray was much better than the clinical machine. The distribution of the kidney and liver vessel image showed (their function) showed the clear vessels structure in the organ. (Figure 2. a, d). The most complex structure of the image was the lung image which was composed of the vessels, capillaries, alveoli and ribs. The thigh was the tumor site and it showed the vessel wind around the tumor (Figure 2. c).

We successfully demonstrated that by coupling proper contrast agents, cellular structure labeled with fluorescent markers can be correlated and imaged with high resolution x-ray microscope. Immunohistochemistry with microtubule cytoskeleton (anti alpha-tubulin, clone number B 5-1-2) was performed with antibody titers of 1:1; 1:100; 1:500; 1:1000; 1:3000; and 1:5000, respectively. EMT-6 cells were seeded on capton and glass slide, staining with DAB/nickel substrate kit and FITC-conjugated second antibody. The fluorescent images were taken by confocal microscope in NSRRC as shown below. The best titers were 1:1000 to 1:3000, these two images could identify the filament structure easily rather than high concentration (>1:100) or low concentration (<1:5000) of first antibody. Tubulin filaments in TXM images correspondent to the same cell observed under confocal microscope subjected to different primary antibody titers were clearly seen.

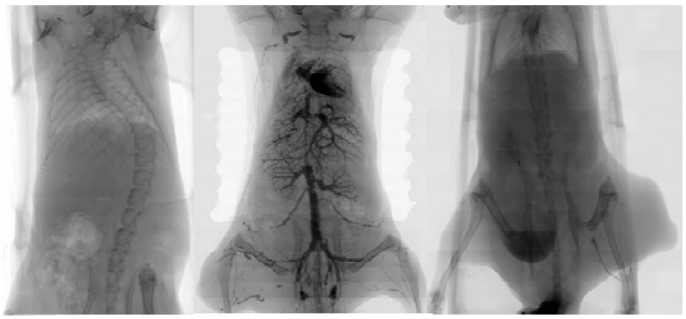

Figure 1. a. the mouse without any contrast agent, it showed clear bone structure. b. the mouse was injected with lipodol® contrast agent, it showed clear vessel structure. c. the mouse was injected with telebrix® contrast agent, the contrast agent diffused in the mouse.

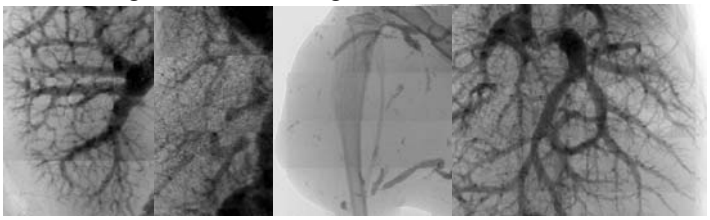

Figure 2. a. Clear kidney vessel with the lipodol® contrast agent**. b.** Lung with clear vessel structure. **c.** Tumor on the leg with nonclear capillaries. d. Liver structure.

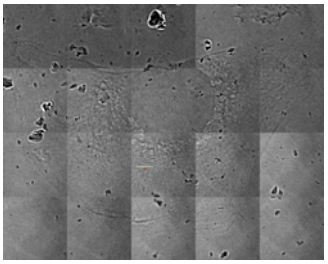

Figure 3. TXM image of α-tubulin specifically stained with Nickel.