International Journal on Magnetic Particle Imaging
Vol 8, No 1, Suppl 1, Article ID 2203087, 3 Pages

Infinite Science Publishing

Proceedings Article

Non-radioactive imaging of bone marrow using antibody-conjugated nanoparticles in magnetic particle imaging

Renesmee Kuo [a,♯], KLB Fung [a,♯], Prashant Chandrasekharan [a,♯*], Quincy Huynh [a], Chinmoy Saayujya [a], Jacob Bryan [a], Kim Hwang Yeo [a], Irati Rodrigo [a], Benjamin Fellows [a], Steven Conolly [a,b]

[a] Department of Bioengineering, University of California, Berkeley, California, USA
[b] Department of Electrical Engineering and Computer Sciences, University of California, Berkeley, California, USA
[♯] These authors contributed equally
[*] Corresponding author, email: prashantc@berkeley.edu

Abstract

Bone marrow serves a crucial role in the body, producing hematopoietic stem cells and blood products. Imaging bone marrow could help doctors determine bone marrow disorders and have an early understanding of the metastatic distribution of tumors in the bone. Colloidal tracers that target the reticuloendothelial system (RES) such as the liver, spleen and bone marrow are commonly used to image bone marrow. Alternatively, antibodies specific to granulocytes, especially neutrophils, can be used to image the myeloid distribution of bone marrow. Using antibody functionalized superparamagnetic iron oxide (SPIO) nanoparticles as tracers, magnetic particle imaging (MPI) could image bone marrow *in vivo*. In this work, we imaged bone marrow *in vivo* using anti-Ly6G antibody functionalized nanoparticles that are specific towards surface antigens expressed on granulocytes.

I. Introduction

The bone marrow is part of the reticuloendothelial system (RES). It is a tissue located within the bones, where immune cells divide, grow and differentiate (figure 1). Hematopoietic stem cells in the bone marrow produce almost all types of blood cells that migrate into the blood stream. Hence, bone marrow is crucial for normal functioning of the body. Inflammation, leukemia and bone metastases can cause bone marrow dysfunction or are a manifestation of it [1], leukemia [2] and bone metastases [3]. Despite the critical role that bone marrow plays in our health, the diseases and disorders of the bone marrow are almost always diagnosed invasively using a bone marrow biopsy, which is a painful and uncomfortable procedure. Moreover, while the cells sampled in the process are very representative for the case of systemic diseases, they do not provide comprehensive information of the marrow tissue. Instead, the information is limited to the sampled region.

Colloidal tracers used in scintigraphy (e.g., Tc99m-sulphur colloid) [4] and magnetic resonance imaging (MRI) (Ferumoxytol) [5] can image bone marrow. Antibody-based scintigraphy tracers that are specific to granulocytes can also be used to monitor bone marrow function, metastases and its metabolic activity. However, the radiation dose from Tc99m or In111 tracers (90 Gy per 10^8 cells [6]) in scintigraphy can possibly cause bone marrow suppression, which eventually leads to bone marrow failure and death [7]. While MRI provides good soft

10.18416/ijmpi.2022.2203087

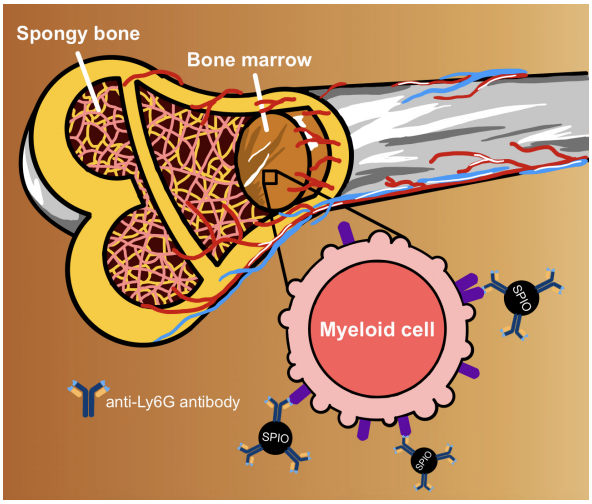

Figure 1: The myeloid progenitor cells expressing Ly6G antigens in murine subjects can be imaged using SPIOs functionalized with anti-Ly6G antibodies.

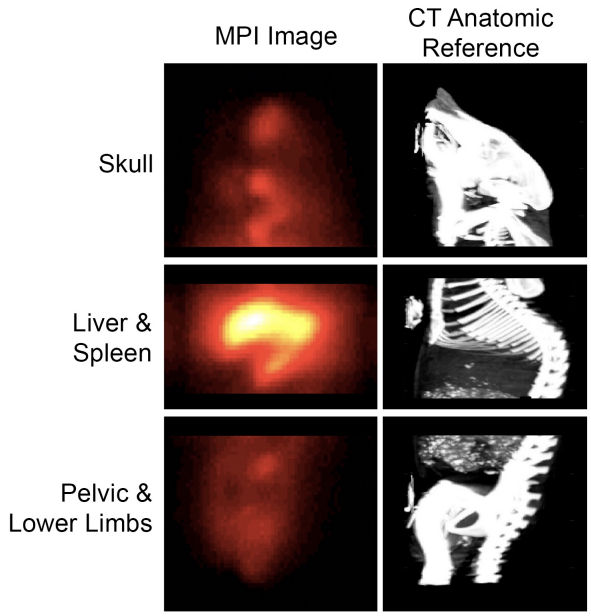

Figure 2: **Immuno-MPI:** 24 hours post anti-Ly6G antibody SPIO tracer administration in a healthy mouse. Anti-Ly6G SPIO tracers predominantly distribute in the organs of the RES, including the liver, spleen and bone marrow.

tissue differentiation and higher spatial resolution, the images are affected by the inherent iron and fat content in red bone marrow, which often ends up requiring multimodal approaches to image the marrow [8]. In addition, the colloids are readily taken up by the RES, which is often overwhelmed by the signal from the liver and spleen.

Magnetic particle imaging (MPI), in contrast, uses non-radioactive and safe superparamagnetic iron oxide (SPIO) nanoparticle tracers with infinite persistence to track cells. It does not affect cell viability or impede cell mobility [9–11]. Hence, MPI can soon safely and effectively image the bone marrow without the pitfalls of white blood cell scintigraphy and MRI. MPI could assist doctors in understanding bone marrow dysfunctions, determining and staging hematological bone marrow disorders.

II. Methods and materials

We used commercially available anti-Ly6G SPIOs (IgG1, REA526 clone, Miltenyi Biotec, GmbH, after dialysis) at a clinically relevant dose (5-5.5 mg of Fe/kg, ~40 µg of protein/mouse). The tracer distribution were evaluated *in vivo* in a healthy mouse model. We used a 6.3 T/m/μ_0 field-free line MPI scanner with 2D projections, a field-of-view (FOV) of 10.6 × 6.2 cm^2 and a 40 mTpp drive field at a frequency of 20.225 kHz. The scanning bed was mechanically translated in the z-direction in 1 mm increments to complete the imaging trajectory for a single projection. The total scan duration was five minutes, 95 s of which were used for data acquisition. 3D tomography images were acquired post-euthanasia. Forty 2D projection images were acquired at equally spaced angles and reconstructed using a Radon transformation. All 3D data were re-binned to a voxel dimension of 0.04 × 0.04 × 0.034 cm^3. The images were compared with a segmented CT image of the mouse [12].

III. Results and Discussion

In general, intravenously administered nanoparticles accumulate in the RES organs, including the liver, spleen and bone marrow [13]. Imaging with anti-Ly6G antibody functionalized SPIOs that bind specifically to Ly6G antigens on murine neutrophils enhances the contrast of bone marrow, as shown in figure 2. This result is similar to anti-NCA95-antibody-based scintigraphy tracers that target granulocytes [14].

The MPI image showed excellent *in vivo* bone marrow uptake of the SPIO tracers, which yields a remarkable contrast of the bone marrow in the skull, pelvic bones, femur and limbs. Hence, MPI could soon be used as a zero radiation functional imaging tool of the bone marrow.

IV. Conclusion

In our previous work, we utilized antibody functionalized SPIOs specific towards granulocytes to image inflammation using MPI [9]. The antibody binds to the antigen expressed specifically on the surface of neutrophils, allowing for tracking neutrophils to lipopolysaccharides-induced inflammation. In this present work, we extended the potential of using antibody-SPIOs to non-

radioactively image the distribution of bone marrow *in vivo*. Showing positive signal and superb contrast in regions with active bone marrow, MPI could help doctors diagnose bone marrow disorders and diseases without harming the cells that originate from hematopoietic stem cells in bone marrow.

Acknowledgments

We gratefully acknowledge support from NIH grants R01s EB019458, EB024578, EB029822 and R44: EB029877, UC TRDRP grant 26IP-0049, M. Cook Chair, Bakar Fellowship, the Siebel fellowship, the UC Discovery Award, the Craven Fellowship from UC Berkeley Bioengineering, NSERC fellowship, as well as the NIH-T32 training and NSF fellowships.

Author's statement

Conflict of interest: Dr. Conolly is the co-founder of a startup company that manufactures and sells preclinical MPI scanners. Ethical approval: All animal procedures were conducted according to the National Research Council's Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee at UC Berkeley.

References

[1] I. Sudoł-Szopińska, E. Kontny, W. Maśliński, M. Prochorec-Sobieszek, A. Warczyńska, and B. Kwiatkowska. Significance of bone marrow edema in pathogenesis of rheumatoid arthritis. *Pol J Radiol*, 78(1):57–63, 2013, doi:10.12659/pjr.883768.

[2] E. A. Sison and P. Brown. The bone marrow microenvironment and leukemia: Biology and therapeutic targeting. *Expert Rev Hematol*, 4(3):271–83, 2011, doi:10.1586/ehm.11.30.

[3] J. Z. Finklestein, H. Ekert, J. Isaacs Hart, and G. Higgins. Bone marrow metastases in children with solid tumors. *American Journal of Diseases of Children*, 119(1):49–52, 1970, doi:10.1001/archpedi.1970.02100050051010.

[4] C. J. Palestro, C. Love, G. G. Tronco, M. B. Tomas, and J. N. Rini. Combined labeled leukocyte and technetium 99m sulfur colloid bone marrow imaging for diagnosing musculoskeletal infection. *RadioGraphics*, 26(3):859–870, 2006, doi:10.1148/rg.263055139.

[5] P. Storey and A. A. Arbini. Bone marrow uptake of ferumoxytol: A preliminary study in healthy human subjects. *J Magn Reson Imaging*, 39(6):1401–10, 2014, doi:10.1002/jmri.24320.

[6] D. A. Goodwin. Cell labeling with oxine chelates of radioactive metal ions: techniques and clinical implications. *J Nucl Med*, 19(5):557–559, 1978.

[7] D. E. Green and C. T. Rubin. Consequences of irradiation on bone and marrow phenotypes, and its relation to disruption of hematopoietic precursors. *Bone*, 63:87–94, 2014.

[8] L. M. Shah and C. J. Hanrahan. Mri of spinal bone marrow: Part 1, techniques and normal age-related appearances. *American Journal of Roentgenology*, 197(6):1298–1308, 2011, doi:10.2214/AJR.11.7005.

[9] P. Chandrasekharan, K. L. B. Fung, X. Y. Zhou, W. Cui, C. Colson, D. Mai, K. Jeffris, Q. Huynh, C. Saayujya, L. Kabuli, B. Fellows, Y. Lu, E. Yu, Z. W. Tay, B. Zheng, L. Fong, and S. M. Conolly. Non-radioactive and sensitive tracking of neutrophils towards inflammation using antibody functionalized magnetic particle imaging tracers. *Nanotheranostics*, 5(2):240–255, 2021.

[10] P. Chandrasekharan, Z. W. Tay, D. Hensley, X. Y. Zhou, B. K. Fung, C. Colson, Y. Lu, B. D. Fellows, Q. Huynh, C. Saayujya, E. Yu, R. Orendorff, B. Zheng, P. Goodwill, C. Rinaldi, and S. Conolly. Using magnetic particle imaging systems to localize and guide magnetic hyperthermia treatment: tracers, hardware, and future medical applications. *Theranostics*, 10(7):2965–2981, 2020.

[11] P. Chandrasekharan, Z. W. Tay, X. Y. Zhou, E. Yu, R. Orendorff, D. Hensley, Q. Huynh, K. L. B. Fung, C. C. VanHook, P. Goodwill, B. Zheng, and S. Conolly. A perspective on a rapid and radiation-free tracer imaging modality, magnetic particle imaging, with promise for clinical translation. *Br J Radiol*, 91(1091):20180326, 2018.

[12] B. Dogdas, D. Stout, A. F. Chatziioannou, and R. M. Leahy. Digimouse: A 3d whole body mouse atlas from ct and cryosection data. *Phys Med Biol*, 52(3):577–87, 2007, doi:10.1088/0031-9155/52/3/003.

[13] C. J. Palestro, C. Love, G. G. Tronco, M. B. Tomas, and J. N. Rini. Combined labeled leukocyte and technetium 99m sulfur colloid bone marrow imaging for diagnosing musculoskeletal infection. *Radiographics*, 26(3):859–870, 2006.

[14] A. Agool, A. W. Glaudemans, H. H. Boersma, R. A. Dierckx, E. Vellenga, and R. H. Slart. Radionuclide imaging of bone marrow disorders. *Eur J Nucl Med Mol Imaging*, 38(1):166–178, 2011.

