Advances in Noninvasive Functional Imaging of Bone

Sheng-Min Lan, MD, Ya-Na Wu, PhD, Ping-Ching Wu, PhD, Chi-Kuang Sun, PhD, Dar-Bin Shieh, DDS, DMSc, Ruey-Mo Lin, MD

The demand for functional imaging in clinical medicine is comprehensive. Although the gold standard for the functional imaging of human bones in clinical settings is still radionuclide-based imaging modalities, nonionizing noninvasive imaging technology in small animals has greatly advanced in recent decades, especially the diffuse optical imaging to which Britton Chance made tremendous contributions. The evolution of imaging probes, instruments, and computation has facilitated exploration in the complicated biomedical research field by allowing longitudinal observation of molecular events in live cells and animals. These research-imaging tools are being used for clinical applications in various specialties, such as oncology, neuroscience, and dermatology. The Bone, a deeply located mineralized tissue, presents a challenge for noninvasive functional imaging in humans. Using nanoparticles (NP) with multiple favorable properties as bioimaging probes has provided orthopedics an opportunity to benefit from these noninvasive bone-imaging techniques. This review highlights the historical evolution of radionuclide-based imaging, computed tomography, positron emission tomography, and magnetic resonance imaging, diffuse optics–enabled in vivo technologies, vibrational spectroscopic imaging, and a greater potential for using NPs for biomedical imaging.

Key Words: Bone; functional imaging; molecular imaging; diffuse optics; nanoparticles.

THE EVOLUTION OF STRUCTURAL IMAGING TO FUNCTIONAL AND MOLECULAR IMAGING OF BONE

Bone is a composite material consisting primarily of hydroxyapatite crystal and type I collagen, which accounts for ~90% of the organic component. Because bone has high mineral content and higher attenuation of x-rays than the surrounding soft tissue, conventional roentgenography was, when introduced in 1895, the first noninvasive structural imaging modality for bone. Because of the advances in physics, digital geometric processing, and computational power, computed tomography (CT) and magnetic resonance imaging (MRI) can process a large array of digital data to reconstruct virtual three-dimensional (3D) images of bone and soft tissue (1). However, functional changes usually precede structural changes in disease process, and molecular signaling aberrations are fundamental to most diseases. Therefore, clinical demands for advanced imaging technology capable of providing functional and molecular information are urgent.

From the functional point of view, physiological bone homeostasis, repair, and pathologic changes are mediated by a balance of positive and negative remodeling processes. Noninvasive functional imaging of bone includes in vivo imaging of bone formation, resorption, inflammation, vascularization, and mechanical properties. At the molecular level, the bone-remodeling kinetics involves a complex molecular interaction network between the cells and the cell–matrix. Appropriate functional and molecular imaging systems with sufficient spatial and temporal resolution provide valuable information to advance bone research and clinical frontiers.

Functional imaging, compared to structural imaging, unravels physiological and pathologic activities, and it has been widely used both in basic medical research and in clinical settings. The scope of functional imaging is shown in Figure 1. Functional imaging senses signals from biological tissue and reconstructs the information into registered images that reflect regional changes in the blood supply, metabolism, chemical constituents, or physical properties. Decades ago, the introduction of intravascular contrast agents not only allowed physicians to distinguish vessels from other tubular structures in the human body but also shed a light on functional imaging (2). Contrast-enhanced CT has been used to assess vascularity

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From the Department of Orthopaedics National Cheng Kung University Medical Center Dou-Liou Branch, Yunlin, Taiwan (S.-M.L., R.-M.L.); Institute of Oral Medicine and Department of Stomatology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University Tainan 701, Taiwan (Y.-N.W., P.-C.W., D.-B.S.); Molecular Imaging Center, Graduate Institute of Photonics and Optoelectronics, National Taiwan University, Taipei, Taiwan (C.-K.S.); Department of Electrical Engineering, National Taiwan University, Taipei, Taiwan (C.-K.S.); Center for Micro/Nano Science and Technology, National Cheng Kung University, Tainan, Taiwan (D.-B.S.); Advanced Optoelectronic Technology Center, National Cheng Kung University, Tainan, Taiwan (D.-B.S.); and Department of Orthopedics, Division of Spinal Surgery, College of Medicine, National Cheng Kung University, Tainan, Taiwan (R.-M.L.). Received October 9, 2013; accepted November 26, 2013. Supported by grants NSC 102-2120-M-006-003-MY3 and NSC 101-2314-B-006-048-MY3 from the Taiwan National Science Council, and a grant from National Cheng Kung University's Headquarters of University Advancement, which is sponsored by the Taiwan Ministry of Education. **Address correspondence to:** D.B.S. e-mail: dshieh@mail.ncku.edu.tw or R.-M.L. e-mail: linrm@mail.ncku.edu.tw

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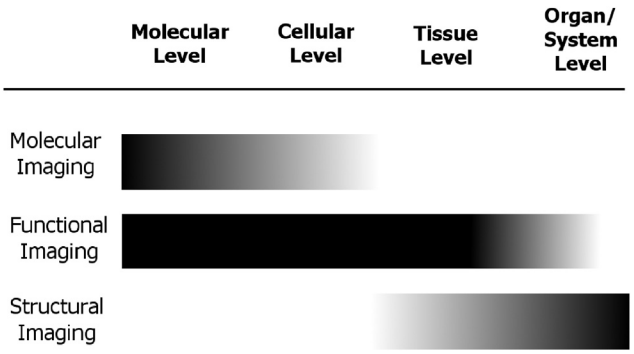

Figure 1. The scope of noninvasive molecular, functional, and structural imaging for the skeletal system. Noninvasive functional imaging reflects changes in the blood supply, metabolism, and chemical constituents or physical properties (electrical or optical), which covers imaging at the molecular to tissue level.

and permeability to distinguish between benign and malignant lesions in orthopedics and other clinical specialties (3–7).

Molecular imaging (8–10), an integral part of functional imaging, is recognized as an important medical advance toward personalized medicine to optimize disease treatment. Detecting subtle functional changes at the molecular level provides advanced diagnoses and clues to therapeutic strategies. The development of molecular probes and new sensing and image processing technologies has contributed to the emergence of molecular imaging. To map the molecular events in vivo, the imaging probes consist of a targeting unit that directs the probe to the site of interest and a reporter unit that emits signals spontaneously or on specific stimulation.

The Targeting Unit of Bone-imaging Probes

Bisphosphonates or diphosphonates, used to treat osteoporosis and similar diseases, preferentially bind to bone (11). Alendronate (a class of bisphosphonates), with a dissociation constant (Kd) of about 10^{-3} mol/L at pH 7 for bone hydroxyapatite (12), is a good targeting unit for calcium-rich tissue. Ozcan et al. (13) synthesized poly(γ-benzyl-l-glutamate) polypeptide-derived NPs by conjugating alendronate and fluorescein isothiocyanate (FITC). After mice had been intravenously injected with such NP, they were found in the bone tissue only ex vivo but could not be noninvasively detected.

A range of distinct glutamate-like receptors and their associated proteins are expressed in both osteoblasts and osteoclasts (14). L-Glutamate binds to osteoblasts ($\sim 10^{-4}$ KD mol/L) (14), and it has been reported (15,16) to be a targeting unit for bone tissue; however, most reports are still limited to in vitro experiments. Because glutamate-like receptors are rich in neurons (17), the practicality of using glutamate as a bone-cell–seeking ligand warrants further evaluation.

Other less reported hydroxyapatite-targeting probes include phosphonate derivatives (18), divalent cation chelating agents such as calcein (19), DCAF (2,4-bis[N,N′-di(carboxymethyl)–aminomethyl] fluorescein) (20,21), tetracycline and its derivatives (20,22,23), alizarin red derivatives (20,23,24), and xylenol orange (20,25). Although the mainstream targeting unit for skeletal imaging is still bisphosphonate derivatives, the reporter unit has substantially advanced in the past decades.

The Reporter Unit of Bone-imaging Probes: From Radionuclides to Near-infrared Fluorophores to Nanomaterials

Radionuclides, with the advantages of highly penetrative γ-rays and being ready to integrate into or with the targeting unit, opened up a wide opportunity for functional and molecular imaging both in clinical settings and in basic research. Despite the merit of high sensitivity and deep tissue penetration, functional imaging using radionuclides harbors the drawback of potential biological hazards and high cost. Therefore, scientists started to search for alternative imaging methods to obtain information from bone. In classical optics, ballistic photons are used to form images, but scattered photons are regarded as noise. The number of ballistic photons decreases exponentially because of the scattering and absorption. The inhomogeneous biological composition of skin, fat, muscle, fibrous tissue, vessel, lymphatics, osseous tissue, and pigments such as cytochromes, hemoglobin, and melanin results in remarkable scattering and absorption of photons, which severely compromises spatially resolved or spectroscopic imaging of deep tissue-like bone.

In addition, visible and ultraviolet lights produce significant autofluorescence in biological tissue, which severely compromises the signal-to-noise ratio. With the advances in photonics and algorithmic modeling of photons absorbed and scattered in turbid media, as in most biological tissue greater than a certain thickness, valuable information can now be retrieved from an array of detectors that collect a plenitude of diffuse photons from the deep photon sources to reconstruct spatially resolved images.

Britton Chance made significant contributions to diffuse optical tomography (DOT) (26–31). As early as 1989, Chance et al. (29) developed a model based on the diffusion approximation to radiative transfer theory. The model predictions, highly comparable to in vivo results and Monte Carlo simulations, are a milestone for diffuse optics. In 2002, Chance et al. (30) developed the amplitude cancellation method to sensitively and accurately detect and locate small objects in turbid media. This breakthrough facilitated the practicality of using nonionizing light emitters in functional imaging in a variety of clinical settings.

Near-infrared (NIR) light has garnered attention because of its biological safety and low tissue absorption coefficient (32). There are two biological windows for NIR: 650–950 nm (NIR I) and 1000–1350 nm (NIR II) (32). The NIR I penetrates the human body very well, and selective NIR spectra are able to differentiate oxyhemoglobin from deoxyhemoglobin to reveal perfusion and oxygenation of deep target organs and to noninvasively study brain cortical