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JCIMCR OPEN ACCESS Journal of Clinical Images and Medical Case Reports

ISSN 2766-7820

Review Article

Open Access, Volume 2

In vivo imaging tools for functional assessment of biomaterials implanted bone regeneration

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Received: Oct 16, 2021

Accepted: Dec 16, 2021

Published: Dec 23, 2021

Archived: www.jcimcr.org

DOI: www.doi.org/10.52768/2766-7820/1504

Abstract

Since the discovery of X-rays and its first use in imaging of a hand, bone tissue has been the chapter of interest in medical imaging. However, X-ray imaging poses limitations nowadays owing to the augmented complexity of implant scaffolds as well as with the advances in bone engineering. As a result, advanced follow-up imaging techniques are of paramount necessity for effective postoperative characterization. Moreover, it is also needed to search for non-invasive, high-sensitivity, and high-resolution structural, functional, and molecular imaging techniques such as acoustic, optical, magnetic, X-Ray, electron, ultrasound, and nuclear imaging, etc. as an alternative to normally used X-ray computed tomography. Further, enthusiastic preclinical scanners have turned out to be accessible, with sensitivity and resolution even superior to clinical scanners, as a consequence helping a rapid transformation from preclinical to clinical applications. Besides, recently, bone-specific probes and contrast agents are developing for better imaging tools in bone-tissue engineering applications. This review highlights such emerging preclinical imaging tools, each with its individual potencies and flaws, either used only or in combination. In particular, multimodal imaging will significantly add to improve the present understanding in the characterization of bone regenerative processes.

Keywords: bone tissue engineering; biomaterials imaging; modern technologies; acoustic; magnetic; nuclear imaging.

Introduction

Bone, the highly vascularized connective tissue, is mainly composed of hydroxyapatite, calcium carbonate, and phosphate which account for two-thirds of the total bone. The rest of the part is composed of collagen, proteoglycans, and other non-collagen proteins including different growth factors and morphogenic proteins with a significant amount of water [1]. These altogether provide the mechanical strength of the bone as well as provide its flexibility. Most of the bones are composed of the outer cortical bone and the inner spongy bone. Bone heals without forming any scar tissues. The process of bone healing may be delayed due to different pathological conditions leading to nonunion, malunion, and other bone infections. Hence, disabled healing conditions sometimes necessitate the application of bone graft. A bone graft may be defined as a material that is implanted for promoting the healing process. The graft material may be used alone or in combination with other materials. The main objective of healing is to bring osteogenesis, osteoinduction, and osteoconduction. Nowadays nanotechnology has been extensively studied in the field of orthopedic and is being used successfully for challenging bone surgery or infections. Nano scaffolds, delivery methods, controlled alteration of surface geography and composition, and bio microelectro mechanical systems are examples of some nanotechnology of applications in orthopedic surgery [2].

The use of nano-technology in biologic research is studied vividly for its unique nature of exceptionally small size, surface functionality and well-matching with cellular components. This technology also helps to upgrade the mechanical strength of scaffold biomaterials to optimize the bone healing responses. Surface modification of the implants has been proved to improve the healing response also [3].

With the advancement of tissue engineering technology, different strategies for nanomaterial construction have also been evolved [4-6]. Hence, these technological applications need to be assessed by varied imaging approaches which will not only be evaluating its morphological aspect but also its functional and molecular information. Amongst numerous technologies, one conventional method like histological techniques lacks detailed information, more preciously in vivo studies [4] and destruction of the samples. To overcome such limitations of conventional technologies, different advanced imaging modalities with the features of noninvasive, longitudinal, and constant supervising of the implant/constructs have emerged and now are used successfully [5-7].

Ideal imaging methods must 1. Determine signals at the subcellular level and enter the whole body and 2. Provide contrast to explore all the vital data including morphological, physiological, and molecular changes [8]. There are, at present, no such ideal techniques that have all these qualities. Hence, the use of different technology is needed and requirement-based. Actually, the imaging tools are functioning based on the interaction of construct/cellular part and imaging energy sources and detect the energy change and emitted by the cells for the formation of an image. Spatial and temporal resolution, diffusion depth, relevant endogenous and exogenous contrast agents, protection, and price are the different parameters that are closely related to modern tissue imaging tolls' specifications [9-12]. Different technologies along with their characteristics and other modalities have been presented in Table 1.

X-ray and Computed Tomography (CT), Positron Emission Tomography (PET) and Single-Photon Emission Computed Tomography (SPECT), Magnetic Resonance Imaging (MRI), Ultrasound Imaging (US), Optical Imaging (OI) are few examples of different modern imaging technologies for assessing tissues/organs/implants, etc. The tissue and implant imaging techniques help the researchers to evaluate the host-graft interaction along with immune reaction against implants and scaffolds. These also help to indicate and follow the signal release cascades [13]. All the available techniques may be divided into two categories; one will provide information on anatomical aspects (CT, MRI, US) and the other will highlight metabolic aspects (PET, SPECT, OI). These modern biomedical imaging techniques greatly increased spatial resolution, infiltration depth, temporal response, recognition understanding, and element specificity along with providing biomaterials-cell contrast and characterization at the interface site [1]. In this review advantages and disadvantages of different technologies along with better visualization of interactions in physical background aimed with need-based, perspective will be discussed. This manuscript will be immensely helpful to the biomaterial and biomedical researchers to choose their required technology for the characterization of tissue-implant interactions, immune reaction to the grafts, implants and scaffolds.

Functional assessment of osteogenic biomaterials

Unlike most other tissues, the bone itself can regenerate and repair itself without forming any scar. There are certain situations where this process may be hampered. Inadequate blood supply, osteomyelitis and soft tissue infections, systemic diseases affecting healing, instability of fracture ends are some of such conditions which may lead to mal or nonunion. Different phases and events of normal bone healing have been presented in Table 2.

Table 1: Different modern imaging technologies with their attributes.

Imaging methods / Properties	Micro-PET	Micro-CT	Micro-MRI	Micro ultrasound	OCT	Optical microscopy	Bioluminescence	Photoacoustics
Imaging depth	Full body	Full body	Full body	10 mm	1-3 mm	0.3–1.0 mm	10 mm	20 mm
Spatial resolution	1–2 mm	5 µm	5-200 µm	20-100 µm	1-15 µm	0.2-1 µm	2-3 mm	50-150 µm
Real-time	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Anatomy	Poor	Excellent	Excellent	Very good	Good	Poor	Poor	good
Endogenous contrast: blood	Poor	Poor	Very good	Good	Poor	Very good	Poor	Excellent
Endogenous contrast: bone	Good	Excellent	Very good	Poor	Good	Very good	Poor	Poor
Blood perfusion	Poor	Poor	Excellent	Good	Poor	Poor	Poor	Excellent
Oxygen saturation	Poor	Poor	Very good	Poor	Poor	Poor	Poor	Excellent
Molecular imaging using contrast agent	Excellent	Poor	Very good	Good	Good	Excellent	Excellent	Excellent
Cost	High	Medium	High	Low	Low	Low	Low	Medium
Portability	Low	Low	Low	High	High	High	High	High
Contrast mechanism	Gamma ray emission	X-ray absorption	Proton magnetization and relaxation	Acoustic reflection (back scattering)	Optical back scattering	Light		Optical absorption
Advantages	Noninvasive, deep penetration, high molecular sensitivity	Noninvasive, deep penetration, high resolution	Noninvasive, deep penetration	Noninvasive, high speed, deep penetration	Noninvasive, cellular-level resolution, high imaging speed	Can observe living cells, and a wide range of biological activity, not affected by electromagnetic field	Noninvasive, *in vivo* studies of infection, cancer progression	Noninvasive, high practical and element sensitivity, deep infiltration

Limitations	Low resolution, radiative labeling	Ionizing radiation, low chemical sensitivity	Expensive, low imaging speed	small resolution and chemical sensitivity, coupling medium required	Superficial penetration, low chemical sensitivity	lacks resolution	May not offer an accurate depiction of the biological outcome, inadequate and wavelength-dependent diffusion of light through animal tissues.	Coupling medium needed
Application areas	Cell metabolism, cell tracking	Engineered bone, pore structures	Fluid content and transport	Mechanics, flow dynamics, scaffold cavitation	Vascularization, cell tracking, scaffold degradation	Biological/cellular events. Gross cellular changes.	Detection of lung inflammation, pulmonary metastasis	Vascularization, oxygenation, cell tracking, cell–biomaterial interaction
Adopted from references [4,9-12,14,15]								

Table 2: Healing events of bone tissue.

	0-5 day	5-10 day	10-16 day	16-21 day	21-35 day
Events	Hematoma [16,17]	Soft callus	Fibrous tissue [18]	Hard callus [16]	Remodeled bone [17]
Major cell predominates	Macrophage, B-cell [19] Stem cells	Chondrocyte, osteoblast 20]	Hypertrophic Chondrocyte [21], osteoclast [22], Macrophage [23]	Myelopoietic and hematopoietic cells [24]	Osteocyte [1]

Different biomaterials and implantable grafts are popularly being used to treat bone healing abnormalities. A perfect bone graft should possess osteoinducting, osteoconducting, and osteointegrating properties for efficient incorporation into the host bone tissue [13]. Osteoconduction is osteoblast stimulation by Bone Morphogenic Proteins (BMP), growth factors and MSCs. Osteoconduction is to provide scaffold bed for new bone growth into. Osseointegration is host bone-implant bonding [25-27]. Assessment of healing is one of the indicative parameters to propose or to expect normal repair of the bone defect. Metabolomics assays like TNF-470 [28], expression of mRNA by in situ hybridization [29], individual mRNA assay [30-33], Micro Array approaches [34-39], evaluation of osteoblastic proliferation including type I collagen, osteocalcin, osteonectin, osteopontin, bone sialoprotein [40], alkaline phosphatase level [45], identification of monoclonal antibodies like SB10 [40] and HOP 26 [41], marking of cells of the osteogenic lineage using retroviral-mediated gene transfer [42], molecular probe-based implant evaluation for modulation of osteogenic process [40,41,43,44] are examples of some common assessment methods which are frequently used to evaluate biomaterials' osteointegration and bone healing.

There are some *in vivo* experimental models like segmental/calvarial bone defect, subcutaneous placement of demineralized bone matrix/material [45-50] and diffusion chamber model [51,52] which are also used to evaluate osteointegration properties of the implanted biomaterials.

Apart from histology, histomorphometry is also used as a valuable tool for the evaluation of bone tissue construct.

Histology

This technique is being used since long back and is successfully been employed to evaluate the attributes and changes of bone cells and scaffolds. Generally, light microscopy is helpful for this reason but the use of a digital pathology slide scanner is the best option for a rapid and high-resolution image. The use of this modified scanner will convert the glass slide into a digital slide and one can see the image at a different real optical image [53]. With the help of this technique tissue morphology, fibrosis, necrosis, inflammatory changes, neo-vascularization, fatty changes, mineralization, new bone formation, bone density, quality, and materials degradation can be measured. Histology of both decalcified and undecalcified implanted bone sections can be performed to assess the bone regeneration at the implantation side as well as to study the activities of biodegradable materials (Figure 1a) [54-56]. In decalcified bone specimens, the microtome cut paraffin embedded and hematoxylin and eosin stained sections are observed in light microscope fitted with a digital camera and connected to a computer for assessing the cellular response with the host bone to the implants [57]. Similarly in undecalcified bone samples, dehydrated, Spurr's resin embedded perpendicular bone sections stained with Masson Goldner's trichrome are observed under light microscope connected with digital camera and computer to evaluate the behaviour of implanted biomaterials.

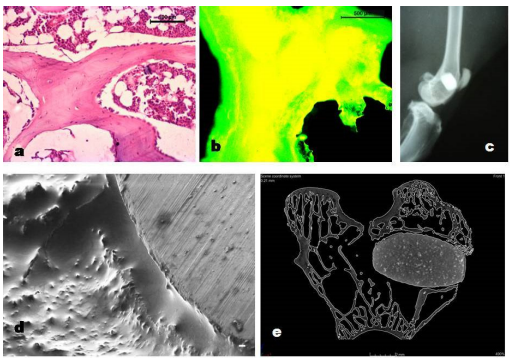

Figure 1: (a). Histology of biomaterial implanted bone (**b).** Fluorochrome labeling of new bone.

Histomorphometry: Histomorphometric examination from bone samples is generally carried out to measure the extent of newly formed osseous tissue, presence of remaining graft particles, and non-mineralized tissue. This robust analysis technique provides quantitative numerical data on bone microarchitecture, remodeling, and metabolism [58]. Histomorphometry also provides an insight into bone properties of certain skeletal conditions like osteomalacia, osteoporosis, etc. although this method provides accurate data, the expense, and requirement of time are the main hindrances. To overcome such problems, a semi-automated quantification method was adopted to measure trabecular area, osteoid area, trabecular thickness, and osteoclast activity using ImageJ toolbox and plugins (Bone J) software [59]. Details of the sample preparation, image capturing, and evaluation was nicely mentioned (Figure 2)[59].

Unlike bone histology, this technique also provides exact disease characterization and response to treatment and information on bone remodeling in a quantitative manner rather than qualitative [60]. It can be useful to the histological figures or to the high-resolution images obtained by different modern techniques. Baddeley *et al*. [61] and Vesterby *et al*. [62] worked on histomorphometry based on stereological formula assuming random and unbiased sampling. Now different techniques of measurement like Bioquant and Osteometrics (www.bioquant.com, www.osteometrics.com.) are available with modern facilities of measurement. Different researchers also used this method for evaluating bone tissue and constructs [63,64]. Pathophysiology of metabolic bone diseases is also well studied by this technology [65-67].

All the modern imaging modalities work on the principles of energy interactions of imaging source with the implanted biomaterials and tissues [14] which mostly includes absorption, scattering and polarization. Depending on the contrast medium, the imaging technologies may be grouped as acoustic, optical, magnetic, X-Ray, electron, and nuclear imaging.

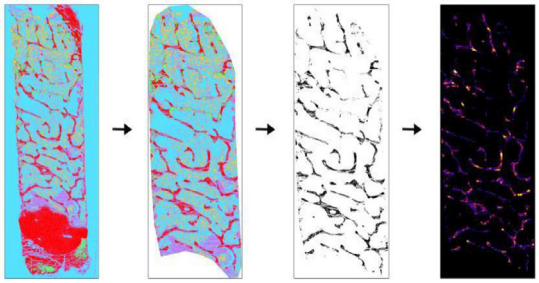

Figure 2: Histomorphometry using Bone J software in the measurement of trabecular bone, thickness etc. [Reproduced Fig. 3 of Ref. 59- open access publication].

Acoustic imaging techniques

Ultrasonography

Three Dimensional anatomical configurations may be obtained from ultrasonographical interventions of the tissues [68,69]. Ultrasonography is the safest imaging method owing to the fact of its lower wave length and higher penetration and may be employed for scaffold material characterization [70] as well as to screen material degradation, and calculate the role and organization of vasculature. The imaging of biomaterials and tissues depends on different attributes of ultrasonography like mass density, modulus, and cavitation. Depending on the above factors, the following modes of application is usually employed to study the intended part; B Mode for mineralization [71], Doppler imaging for vascular study [72], ultrasonic elastography for scaffold degradation [69], microbubble mediated sonography for the study of drug delivery or gene therapy [73], plain ultrasonography, etc.. This technique lacks of radiation and is of low cost with the facility of portability.

Optical

Optical Imaging (OI) is the oldest technique with high sensitivity that can create 2D images by passing visible light through thin objects. This modality lacks better depth visualization. This procedure works on the principle of photon detection. Most of biomedical laboratories prefer to use this modality owing to its flexible imaging contrasts and high spatial resolutions. Following are the different types of OI techniques

Fluorescence imaging (FLI): Fluorescent microscopy methods allow assessing cellular relations, tissue function, and in some cases helps to examine tissue engineered biomaterials in situ with the use of fluorophores and without the necessity of exogenous labels. Different fluorescent dye for imaging bone regeneration is commercially available like high-affinity bisphosphonate-based bone agents and tetracycline derivatives targeting bone [1]. One of the fluorescent marker is tetracycline that follows the ionized calcium and are deposited the site of active bone mineralization during the healing process. New bone formation at the implanted site can be seen with bright golden yellow fluorescence in the green background (Figure 1b). This can be assessed using a Fluorescence microscope connected with digital camera, computer and source of U-V light. The new bone formation can be quantified by measuring the golden yellow pixels inbuilt with the computer system. During the imaging process, the sections should be placed close to the surface and/or set in a particular optically translucent window chamber. Hydrogel-based scaffolds are predominantly a difficult subject for imaging owing to their elevated water content. However, alteration of hydrogels with a fluorescent tag allows screening of degradation of in vitro and subcutaneously implanted *in vivo* bioimplants [74].

Fluorescence molecular tomography (FMT): Cathepsin K targeting probe is used to target osteoclastic activity [75]. Using this technology, it is likely to quantity each discrete source detector-pairs at the cost of an extended measurement time. [76,77]

Bioluminescence imaging (BLI): Bone repair is measured by the use of luciferase-bearing transgenic Mice [70,78]. Through this technology, it is easier to evaluate transgene expression, progress of infection, tumor progress and metastasis, transplantation, toxicology, viral infections, and gene therapy [79]. De´gano et al., used this technique to assess the in vivo bone regeneration ability of implanted human bone marrow and adipose tissue MSCs, loaded arginine-glycine-aspartate crosslinked hydrogel scaffold in mouse calvarial defects over a period of 12-week. He observed that luciferase-labeled cells could be monitored in vivo for a prolonged period. [78]

Confocal microscopy (CM): It is mainly used for imaging of tissue and scaffold materials [80]. Using this technology in the second near-infrared (NIR-II) window, it has been established that bone is a vital organ for the retention of nanoparticles. Small polymer nanoparticles of ~15 nm diameter displayed fast buildup and long-standing retention in bone. This technique helps to identify the dispersion of nanoparticles in the endothelial cells of sinusoidal vessels in bone marrow [81]. In modern machines technology like NIR-II *in vivo* imaging system equipped with an 808-nm laser and an InGaAs camera (Photonic Science, UK) is available where emission is collected with 1319 nm long-pass filter.

Multiphoton microscopy (MPM): This technique can be used for biomaterial and tissue visualization [80], and cells of bone marrow [82]. Multiphoton microscopy can be employed even without adding fluorescent molecules to enable cells to be imaged like many other approaches. For imaging of biological samples, a femtosecond laser must be used. Multiphoton microscopy is a commanding tool for high-resolution imaging in a 3D sample that is optically opaque. It also helps to visualize the in vivo vibrant movement of osteoclasts and osteoblasts, in addition to their interactions with each other [83]. To understand the mechanism of interactions between implanted biomaterials and vascular micro atmosphere in a cranial bone defect window

chamber mice model, multiphoton laser scanning microscopy is a useful technique that offers high-resolution, four-dimensional imaging analyses [84]. The same imaging tool was successfully used to assess the osseous tissue regeneration on BMSC-mediated calvarial bone defect repair [85,86]. Another advantage of this technique is that it permits to take chronological in vivo images of bone tissue–engineered constructs with high temporal and spatial resolution for more extensive periods without disturbing the biological phenomena [87].

Photoacoustic tomography (PAT): Measures *in vivo* oxygenation saturation of hemoglobin along with evaluation of tissue angiogenesis [5,88]. Tissues are irradiated by pulsed laser light which ultimately leads to production of pressure waves caused by temperature and volume. Generally, NIR and visible light is used in PAT [89].

Intravital microscopy: Intravital microscopy is a visualization of individual cells in living condition [90]. It mandatorily needs implantation of imaging window in the animal under study [91]. The key benefit of this technique is that it can visualize the cells in living mode and the cellular activities can be recorded in real mode. If the image resolution is high, it is practically possible to make 3D images of individual cells through this technique. Specific fluorescent labeling is necessary to record the cellular events by this process. The main disadvantage of this technique is that it cannot visualize all cell types as the distinguished fluorescence levels are not available for all the cell types. Osteocyticosteolysis progressions in bone have been documented by Eleonora *et al.* [92] and Hiroshige *et al.* [93] by using this technology. Hemodynamics and Vascular Permeability of mouse bone marrow were studied by Yookyung *et al.* [94] with this modality. The role of microvasculature for bone healing in normal and perturbed bone was documented by Winet [95] with the help of this technique (Figure 3).

To track peri-implant endosseous healing, Intravital imaging plays a vital role to calculate angiogenesis and perivascular cell dynamics that happens around orthopedic and dental implants. This new imaging technique has been used to assess bone angiogenesis and cellular dynamics during bone defect repair [86,96,97]. However, scanty information on high-resolution in vivo longitudinal reports is available to study peri-implant angiogenesis. Of late, Khosravi et al., 2018 developed a novel technique to quantify the vascular and cellular changes around the bone implants using an intravital imaging approach [98]. This technique can also be employed to as imaging tools for ectopic bone formation in bone-tissue regeneration [84].

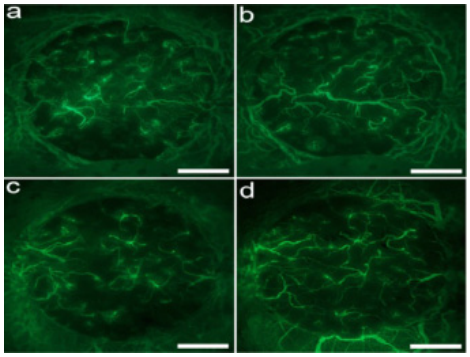

Figure 3: Intravital microscopy of Fluorescence images shows vessel generation at the BCP implanted cranial window defect area using intravenous injection of FITC dextran. (a, c-at 14 days; b, d-at 28 days) [*Reproduce eCM journal with acknowledgement-Open access paper Ref. 53*]

Optical coherence tomography (OCT): It measures the structural changes of the scaffold in the 3D mode which might be due to degradation of scaffold or matrix deposition [99]. It is based on low-coherence interferometry, characteristically using near-infrared light.

X-Ray, electron, and nuclear imaging of material structures at several scales

Conventional X-ray

Among the diverse non-invasive characterization parameters, the conventional X-ray study is one of the best methods to assess the bone-implant interaction as well as mechanical interlocking (Figure 1c) [55]. This technique also provides valuable insight into the degradation kinetics of implanted scaffold within the bone defect [54]. The gradual reduction of radio-opacity states degradation of implants vis-à-vis new bone tissue regeneration [100]. Although X-ray-based imaging provides excellent resolution, and is very fast, however, needs ionizing radiation and thus can potentially damage implanted implants. Phase contrast x-ray shows better sensitivity to image polymer scaffolds [101].

Electron microscopy (EM)

It consists of Scanning Electron microscopy (SEM) and Transmission Electron microscopy (TEM). It is the best modality that can provide the highest resolution image of cell-scaffold interface. These images can provide details like scaffold pore size, fiber orientation, cell deposition, and interface interactions. It actually used for nanoscale characterization of biomaterials. This technology is costly and very limited capacity to provide images of the specimens containing live cells **(**Figure 1d) [102].

Nuclear imaging: It is one type of ionizing imaging modality that detects photons emitted from either isotope or from radiotracers [103]. This process does not produce any toxicity. It can penetrate deep tissues even a whole-body scan is also possible. It can detect radio-labeled cells or tissues or substances at the nano or pico-molar level. Nuclear imaging has poor spatial resolution capacity owing to the scattering of gamma rays by tissues; hence MRI or CT is advised in association with gaining better architectural and molecular information. It includes Positron Emission Tomography (PET) and Single-Photon Emission Computed Tomography (SPECT).

SPECT: In this system commonly ^{99}mTc, ^{111}In, ^{125}Igamma (γ) emitting radioisotopes are used [104]. This modality uses multiple energy windows at the same time, hence different radioisotopes labeled tracers may be injected simultaneously. Diphosphonates labeled with ^{99}mTc radioisotopes have a longer half-life than any other used in nuclear imaging, thus more easily to trace the healing process of bone by much fewer periodical injections of radiotracers.

PET CT: It is generally employed to monitor the cell metabolism using a radioactive glucose analog ($_{18}$F-fluorodeoxyglucose). In this scanning modality, two oppositely directed annihilation photons by positron (β+) emitting isotopes are identified over time concurrence by a pair of detectors. Dynamic PET Compartmental analysis enables to absolutely quantify the tracer bone uptake and can evaluate the comparative osteoblastic activity [105,106]. The cost of PET instrumentation is much more due to the establishment of a cyclotron unit for production of radioisotopes. CT scan generally offered standard anatomic information whereas PET illustrated the higher new bone formation at the

implanted defect site. Since bone regeneration encompasses a vibrant interaction of biological processes, dynamic PET-CT imaging is thought to be an appropriate means for real-time tracking of the amount and rate of bone formation.

In nuclear imaging, the most common disadvantage is that the radioisotopes which are generally used have a very short half-life hence repeated injections; once per week, are generally necessary to trace or track the events of bone healing. Another drawback is its inability to differentiate the osteoblastic activity between scaffolds induced with host tissue made [8,106]. To summarize, PET imaging provides higher contrast of the osteoblastic activity at the defect area as compared to SPECT, which might be due to the better spatial resolution of PET scanners.

Micro CT

Micro-CT (mCT) has been utilized to illustrate the internal organization of a broad range of engineering scaffolds before use at *in vivo* situations. This technique is the most accepted method of characterization of tissue engineered scaffold implanted bone owing to its highly absorbing properties of mineralized tissues. It can measure the details of internal structure of bone at both macro and micro levels**.** Here subjects are scanned in different angles and ultimately converted to a 3D image (Figure 1e). Bone density, surface area details, vasculature, osteocytic identification all can be measured by this noninvasive method in conjunction with contrast agent and synchrotron-radiation micro-CT. Factors like movement artifacts and cumulative radiation are to be considered while using this technique [70,107,108].

Magnetic imaging: Magnetic Resonance Imaging (MRI) is employed for the characterization of fluid [109] and hence very challenging to image the bone as its water content is less. MRI is usually employed to monitor cartilage [110], adipose tissue [111], vascularization [112] and exo or endogenous contrast [113]. Application of MRI for tracking of stem cell differentiation and degradation [114–116] and *in vivo* biomaterials interactions study in small animals [117] is also evident in the literature. In MRI different imaging sequences are used like T1 and T2 weighted. It varies depending on the part under investigation. In MRI, T1 weighted sequences are commonly employed for adipose tissue, blood moving at slow speed, paramagnetic contrast media which are formed by short echo (TE) and repetition (TR) times. In contrast, T2 uses longer TE and TR. As the bone contains no free protons, bone appears black as gives no signal. Thus modifications of this modality to semi-quantitative MRI approaches have been evoked for evaluating hard tissues which are as follows a. Nuclear Magnetic Resonance (NMR) spectroscopy b. Ultra-Short Echo Time (UTE) c. Zero Echo Time (ZTE) d. Sweep Imaging With Fourier Transformation (SWIFT) [70,107,108].

Conclusion

Current biomedical imaging modalities have reformed the research with images of detailed architectural, operational and molecular information of tissue constructs having a high spatial resolution, deep infiltration, greater temporal sensitivity, and better elemental specificity. More and more developments in the field of instrumentation and techniques are ongoing for characterizing bone scaffold materials along with the host tissue reactions against the construct. Previously, unattained tissues or biomaterials can be visualized with different technologies. Of late, much more modifications again strengthen the responsiveness of tissue under study by application of CLARITY and Expansion Microscopy (ExM). In CLARITY tissue clearing techniques are employed for better clear resolution whereas in ExM tissues are physically expanded 4-5 times for attaining higher resolution. In spite of remarkable progress in imaging technologies, several considerations for further developments have to be taken into considerations like super-resolution techniques in the US, improved depth in OI, technologies for accelerated imaging with high resolutions in X-ray, and nuclear imaging modalities. The growth and developments in the sector of biomedical engineering will ultimately help the researchers to select the appropriate tissue construct with more detailed knowledge and skill.

Declarations

Acknowledgement: The authors acknowledge reproducing one figure (Figure 3 in this manuscript) from the www.ecmjoural.org and article figure no 3 of Wernike et al 2010, 19 http://wap.ecmjournal.org/journal/papers/vol019/pdf/v019a04.pdf. The authors also acknowledge the kind support of the Vice-Chancellor, West Bengal University of Animal and Fishery Sciences, Kolkata, India.

Conflict of interests: The authors declare that there is no conflict of interest in this manuscript.

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