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Integration of Biophotonics with Bone-on-Chip Technology for Continuous, Non-Invasive Monitoring of Bone Regeneration

Imanda Jayawardena[1*], Stefan Andersson-Engels[1,2] and Rekha Gautam[1]

**imanda.jayawardena@tyndall.ie*
1. Tyndall National Institute, Lee Maltings Complex, Dyke Parade, Cork, Co Cork T12 R5CP, Ireland.
2. Department of Physics, University College Cork, Cork, Co Cork T12 K8AF, Ireland.

Abstract

The need for non-invasive, real-time, continuous monitoring tools in bone regeneration is essential to improve early diagnosis and therapeutics. Bone-on-chip (BOC) platforms which replicate physiological microenvironments are a useful component within this context. Their integration with biophotonics-based imaging techniques marks a significant advancement in preclinical bone research. For the first time, this review explores how biophotonics can be utilised to improve the accuracy and efficiency of BOC-based studies. As the demand for predictive models that closely mimic bone healing increases, BOCs offer a robust alternative to traditional in-vitro and in-vivo models by combining microfluidics and advanced biomaterials to mimic native bone physiology. We discuss a range of optical methods; including Raman spectroscopy, optical coherence tomography (OCT), second harmonic generation (SHG), and diffuse correlation spectroscopy (DCS), which improve the spatiotemporal resolution of osteogenic processes. Additionally, photoacoustic imaging and near-infrared spectroscopy (NIRS) facilitate deep tissue penetration and vascular assessment. Incorporation of artificial intelligence (AI) and machine learning (ML) within BOC platforms enable automated, high-throughput analysis of real-time datasets, for optimised bone regeneration. Collectively, this review highlights how biophotonics, advanced biomaterials and computational modelling improve the translational potential of BOCs. By establishing multimodal, data-driven monitoring methods, these platforms offer strong potential for advancements in preclinical research and therapeutics development.

Keywords: Bone, Osteogenesis, Bone-on-Chip, Organ-on-Chip, Biophotonics, Non-invasive Monitoring

1. Introduction

BOCs are three-dimensional (3D) microfluidic systems designed to replicate the physiological environment of bone tissue. They offer a more accurate and controllable alternative to traditional preclinical models, that are used to study bone regeneration and disease mechanisms (Figure 1A). However, accurately monitoring bone healing and regeneration remains a major challenge in BOC models and bone tissue engineering. To ensure clarity, this review uses the following definitions for key monitoring terms given in Box 1.

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Box 1. Defining Key Monitoring Concepts

- **Non-invasive Monitoring:** Techniques that assess tissue without causing physical disruption. In the context of BOCs, this refers to monitoring cellular processes without terminating the experiment or altering the microenvironment. Clinically, it refers to methods that do not require surgical incisions or tissue removal.
- **Label-free Imaging:** Methods that generate image contrast from intrinsic optical properties of molecules and tissues (e.g., scattering, absorption, or nonlinear responses), eliminating the need for external fluorescent labels, dyes, or contrast agents. This avoids potential issues such as phototoxicity and alteration of natural cell behaviour.
- **Real-time Monitoring:** The ability to acquire and display data with minimal delay, allowing immediate observation of biological events as they occur.
- **Continuous (or Longitudinal) Monitoring:** The capacity to perform repeated measurements on the same sample over extended periods (hours to weeks). This enables tracking of dynamic processes, such as cell differentiation, matrix deposition, or healing trajectories over time.

While several reviews have addressed BOC technologies and their applications in bone research[1–13], and a few have focused on monitoring bone regeneration[13–18], a comprehensive review that strategically merges the fields of BOCs and advanced biophotonics for real-time, non-invasive evaluation is currently lacking. Conventional monitoring methods often rely on invasive procedures, endpoint analyses, or techniques that lack biomolecular specificity or involve ionising radiation, limiting their translational value (Figure 2). This review addresses this critical gap by focusing specifically on how a diverse suite of photonics-based methods can be integrated into BOC platforms to enable continuous, high-resolution monitoring of osteogenesis. We discuss how the integration of optical imaging, microfluidics, sensors, nanomaterials, multimodal diagnostics, and AI-enhanced data analysis within BOC systems overcomes current limitations in assessing osteogenesis and bone–implant integration (Figure 3). This convergence of organ-on-chip (OOC) platforms with advanced imaging presents a timely opportunity to advance bone healing related research.

Despite advancements in surgical techniques and materials, a global cost of US$664 million and an annual growth rate of 13%, implants and bone grafts continue to have significant failure rates exceeding 40%, depending on implant type and site[19]. For instance, dental implant failure rates range from 5% to 10% and are influenced by factors such as surgical skill, patient health, and material used. In orthopaedic implants, long-term failure rates can vary between 5% and 20%, depending on implant type and physiological conditions of the patient[20,21]. Infection is the key reason for implant failure, with peri-implantitis affecting up to 20% of dental implant recipients. In orthopaedic surgery infection rates can reach 4%, often requiring implant removal and revision. Autografts have been most effective in bone grafting, with failure rates ranging from 5% to 15%, as opposed to synthetic grafts with failure rates from 20% to 30% owing to poor integration and resorption.

Implant and graft failures can lead to prolonged pain, recovery and increased healthcare costs. A major contributor to these outcomes is the lack of real-time, continuous monitoring, thus complications such as non-union or infection go undetected until they become clinically significant. This highlights the need for advanced biomaterials that are capable of producing

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infection-resistant implants and methods to continuously assess bone healing. In this context, tissue engineering methods, particularly when integrated with OOC platforms and novel imaging modalities could offer suitable solutions. However, despite the progress of BOC technology (Table 1) and optical techniques (Table 3), there has not been a comprehensive review to date that addresses strategic merging of the two fields for real-time, non-invasive evaluation of bone regeneration. Thus, our review addresses this gap in the literature, evaluating current and emerging osteogenesis monitoring techniques. Emphasis is placed on BOC systems as physiologically relevant in-vitro models and photonics-based methods for non-invasive, real-time assessment. By highlighting these advancements, the review aims to contribute to reducing failure rates and earlier detection of complications, ultimately minimising their clinical and economic impact.

2. Emergence of BOC Models and Their Clinical Applications

Bone grafts used in tissue regeneration are broadly classified by origin: autografts, allografts, xenografts, and synthetic grafts. Autografts, harvested from the patient remain gold standard due to their osteogenic, osteoinductive, and osteoconductive properties, but are limited by donor site morbidity and availability. Allografts from human donors are more readily available, however, integrate slowly, while xenografts from other species contain immunogenic and infection risks. Synthetic grafts, composed of metals, ceramics, polymers, or their composites, provide tunable properties and are increasingly considered as suitable alternatives[19,22–26].

Bone tissue engineering comprise of osteoinductive signals that promote differentiation, and osteoconductive scaffolds that provide structural guidance for new bone, where, despite progress, integration and biological functionality remain key challenges[23]. Vascularised grafts and integrated cell therapies have shown promise, however, require a stronger understanding of bone biology for clinical success. In this regard, BOC platforms have emerged as improved alternatives, replicating the bone microenvironment in a controlled and physiologically relevant manner[2,26,27]. BOC systems provide a platform for testing and optimising new materials and help develop more biocompatible, mechanically robust platforms for promoting bone regeneration (Figure 1A). These microfluidic-based models allow finer control over cellular, biochemical, and mechanical cues, for more predictive simulations of in-vivo bone physiology and responses.

Tables 1 and 2 and Box 2 outline advancements in BOC models, label-free monitoring and provide examples of how BOCs are clinically used. An overview of this is also presented in Figure 1.

Figure 1. Example Applications of BOCs
(A) Bone remodelling, disease modelling, tissue regeneration, and drug testing. A: A trabecular bone organoid model that reproduces coexisting active and resting bone surfaces using demineralised bone paper (DBP). B: A microfluidic device to study neuron–breast cancer crosstalk in bone metastasis. Selective and dynamic multicellular paracrine signalling was observed between sympathetic neurons, bone-tropic breast cancer cells, and osteoclasts. C: A microfluidic 3D printing- responsive scaffold with biomimetic enrichment channels for bone regeneration. D: A biomimetic BOC platform for high-throughput osteoporosis drug testing was assisted by artificial intelligence image analysis. Reproduced from reference [2] with permission from American Chemical Society, copyright 2023.

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(B) A vascularised human bone marrow-on-chip was developed with optically clear poly(dimethylsiloxane) (PDMS) channels. In the top channel, hematopoietic stem cells ($CD34^+$) were seeded, while endothelial cells (EC) created a vascular lumen in the bottom channel. After 2 weeks of In Vitro culture, hematopoietic stem cells differentiated in multiple blood cell types (magenta: erythroid lineage; yellow: megakaryocyte lineage; blue: neutrophil and other haematopoietic lineages). Scale bar, 20 μm. Reproduced from reference [28] with permission from MDPI, copyright 2021.
(C) Construction of arthritis model on-chip. Immunofluorescence presented the expression of aggrecan, collagen type I, and collagen type II, matrix metallopeptidase 13 (MMP13) expression was up-regulated in the hyperphysiological compression (HPC); The balloon inflated by pressurised air supplied the compression (upper channel), 3D laser scanning microscopy measured the deformation (lower channel). Reproduced from reference [9] with permission from Elsevier, copyright 2023.
(D) Design, flow dynamics simulations, and prototyping of the microfluidic device. 3D height map of the PDMS device, produced using soft lithography on the silicon moulds, and optical microscopy image of the device hosting two 3D bone models. Reproduced from reference [28] with permission from MDPI, copyright 2021.

Table 1. Advancements in BOC Models

Advancement	Description
Microfluidic Systems and Biomimetic Scaffolds	Microfluidic BOC systems (Figure 1D) are typically coupled with biomimetic scaffolds such as hydrogels or hydroxyapatite composites that mimic the extracellular matrix (ECM). These scaffolds provide mechanical cues that support cellular attachment, proliferation, and differentiation[29–31]. Modulation of biochemical and mechanical cues are also possible for studying osteogenesis, drug responses, and disease mechanisms[3–5,32,33].
Advanced Microfabrication	Techniques such as 3D printing and lithography (Figure 1A, D) allow spatial control over cell distribution, ECM composition and mechanical stimuli[6,24,34,35]. Bioinspired scaffolds replicate the ECM environment to support osteogenic differentiation and mineralisation[7,8,35–37].
Mechanobiological Stimuli	Mechanobiological inputs such as cyclic loading and fluid shear stress enhance osteocyte activity and ECM deposition, more accurately replicating in-vivo conditions[2,9,38,39]. Dynamic stimuli such as compression and tension help study mechanotransduction[34,39]. These are delivered through microfluidic bioreactors that provide perfusion and nutrient exchange for optimal cell viability and differentiation[34,35,40,41].
Computational Modelling	Computational modelling; including finite element analysis and computational fluid dynamics, aids in designing complex microenvironments and predicting clinical performance[2,29]. These models are also helpful for evaluating scaffold designs prior to fabrication, ex-vivo testing and in-vivo implantation, saving time and cost through virtual prototyping and optimisation.

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Controlled Release of Biochemical Factors	The incorporation of controlled-release systems for growth factors such as BMPs, PTH, and vascular endothelial growth factor (VEGF) allows targeted regulation of bone cell activity and mechanotransduction studies[6,29,35,42].
Vascularisation and Immune Integration	Early BOC limitations involving vasculature have been addressed using endothelialised microchannels and perfusable scaffolds, improving angiogenesis and nutrient exchange[4,11] (Figure 1B). Co-culturing osteoblasts with endothelial cells has improved osteovascular modelling[7,9], while immune system components have been integrated to simulate conditions such as osteomyelitis and osteoporosis[1,43].
Nanomaterials	Engineered nanocomposites and nanoparticles are increasingly being utilised to enhance mechanical properties of scaffolds and locally deliver growth factors for improved regeneration[3,32,44–46].
Multi-Organ-on-Chip Integration	Multi-organ-on-chip systems combining BOC platforms with models of other tissues (e.g., liver or vascular networks) support systemic pharmacokinetic studies and study of inter-organ crosstalk during bone regeneration and disease progression[31,47].

Box 2. Advancements in Label-free Monitoring on BOC Platforms

Sensors

Implantable and flexible sensors have been incorporated into BOC platforms to measure mechanical strain, biochemical signals (e.g., pH, calcium, alkaline phosphatase), and microenvironmental conditions during healing. These sensors help assess mechanical integrity, alignment, osteogenic activity, and mineral deposition[9,1,2,48–53]. Remote and real-time monitoring is conducted via wireless sensors using radio frequency identification (RFID) and piezoelectric transducers[54–56]. Piezoelectric materials or conductive hydrogels based platforms allow in-situ monitoring of cellular and tissue activity, for improved clinical decision-making[45,46,57]. Flexible piezoelectric ultrasonic systems are applied as wearable sensors to non-invasively monitor patient-specific loading and bone density[35].

AI, ML, and Internet of Things (IoT)

ML and IoT technology are used to process large imaging and sensor platform datasets, predict healing trajectories, identify therapeutic responses and personalise treatment methods[58,59] (Figure 2.1 A). AI algorithms automate image quantification and recognise subtle patterns in osteogenic differentiation[38,60,61]. A notable case study is the high-throughput BOC platform for osteoporosis drug testing developed by Paek et al. (2023)[60]. In this system, AI-based deep learning algorithms automatically analyse fluorescence microscopy images to quantify osteogenic differentiation and the nuclear translocation of β-catenin, a key marker in bone formation pathways. This allows rapid, objective, and high-content screening of drug efficacy, a task that would be prohibitively laborious if performed manually. In another example, machine learning and neural networks were used to develop a vascular network quality index (VNQI) that quantitatively compares blood capillaries-on-a-chip, achieving over 94% accuracy in relating vessel morphology to function[62]. Beyond image analysis, these tools also contribute to the optimisation of biomimetic scaffold architecture to ensure better host integration[63]. ML techniques

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support the analysis of multi-parametric datasets to identify early indicators of bone formation and remodelling[64,65]. As discussed in recent reviews, the synergy between AI-based optical biosensing and microfluidic integration is rapidly advancing and addresses core technological challenges[66,67]. Microfluidic devices generate vast quantities of complex data, particularly from imaging and spectroscopic readouts. AI methods, especially deep learning algorithms such as Convolutional Neural Networks (CNNs), possesses the analytical power to process this data deluge. AI models can automatically de-noise complex Raman spectra, extract subtle molecular fingerprints from high-background signals, and classify cellular or molecular states with impressive and beyond-human accuracy and speed. For instance, in drug development, AI-enhanced Raman spectroscopy can monitor drug-biomolecule interactions in real-time within an OOC, while in diagnostics, it can detect disease biomarkers at much earlier stages than conventional methods. This integration converts the labour intensive, error-prone process of manual spectral analysis into an automated, intelligent, and highly sensitive[66–68]. Ultimately, predictive models powered by AI can simulate complex biological processes such as fracture healing and treatment responses and help guide the development of personalised therapeutics by integrating patient specific data with experimental results[18,69–71].

Table 2. Examples of Clinical Applications of BOCs

Application	Examples
Drug Screening and Disease Modelling	BOCs are increasingly found to be clinically relevant in drug screening and disease modelling (Figure 1A). Their ability to simulate the native bone microenvironment permits physiologically accurate osteotoxicity testing and high-throughput drug screening, thus enhancing preclinical research validity over conventional 2D or animal models[72,73].
Oncology and Bone Disease	In oncology, BOC systems have been used for bone metastasis modelling and anti-metastatic therapy that prevent tumour cell colonisation in bone tissue[74,75] (Figure 1A). Similarly, they provide understanding of bone remodelling in osteoporosis and osteoarthritis by simulating osteoblast–osteoclast interactions and responses to mechanical and pharmacological stimuli[76,77] (Figure 1D). These systems have led to the development of novel treatments beyond mere pain management, including 3D bioprinting of cartilage, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based gene editing for osteogenesis imperfecta (OI) and stem cell-based, nanoparticle-enhanced treatment to improve bone density and regeneration[4,12,78,79].
Personalised Therapeutics	Personalised BOC models have facilitated development of patient-specific therapies. Autologous stem cell seeded systems have been used to simulate responses to grafts in large bone defects to improve therapeutic outcomes, while reducing trial-and-error in clinical practice[80]. Gradient scaffolds in BOCs replicate cartilage–bone interfaces for targeted osteoarthritis and joint injury therapies[81]. In craniofacial applications, dissolvable 3D-printed moulds populated with patient-derived cells have facilitated the development of custom implants with improved implant integration and reduced surgical

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	complications[82]. BOCs have also been used to investigate bone remodelling in microgravity conditions, shedding light on space-induced bone loss and aiding the development of treatments for osteoporosis on Earth[4].
Orthopaedic Applications	In orthopaedics, BOCs are applied to create disease models reflective of an individual's genetic and physiological profile for real-time simulation of therapeutic responses[50,83] (Figure 1C). These systems also evaluate novel biomaterials and stem cell therapies for defect repair, however still rely on imaging methods such as micro-CT and MRI, which are limited as outlined in Section 3[84–88]. Biosensor integrated BOCs provide readouts of bone strength and fracture healing under physiological loads, leading to the development of advanced orthopaedic implants and accurate monitoring of implant performance[79,89].

Taken together, the convergence of microfluidics, sensors, automated methods, and patient-derived models within BOC platforms provide in-vitro systems for drug development and mechanistic studies, and offer a foundation for personalised and targeted therapies for bone disorders to improve patient outcomes[5,90].

However, a challenge that still remains is the lack of robust, non-invasive, label-free and continuous monitoring tools to assess implant integration and osteogenesis in real-time. Biophotonics-based imaging methods that offer these features are therefore essential components of future BOC platforms. The following sections explore current imaging methods and emerging biophotonics-based techniques designed to meet these needs.

3. Current Methods in Monitoring Bone Healing

This section provides an overview of key traditional methods used monitor bone regeneration[18,91] some of which are given in Figure 2.

Histology and Histomorphometry

Early methods of monitoring bone healing, that are still in use, such as histology and histomorphometry provide information into the levels of new bone formation induced by graft materials (Figure 2A). These methods enable examination of tissue responses such as inflammation, fibrosis, and graft integration with the host bone. Through histological analysis, the extent and quality of newly formed bone could be assessed through parameters such as bone, cartilaginous, fibrous callus areas and micro-vessels in callus areas. However, histology has limitations as it is an endpoint test that requires invasive sampling and laborious sample preparation[92–94].

Biochemical Markers

The use of biochemical markers, which include specific proteins and enzymes associated with bone metabolism, complement imaging methods by providing information on metabolic and cellular activities during healing. Elevated levels of these markers can act as indicators of bone turnover healing progress[95]. Similar to histology, the use of biochemical markers requires the use of biopsies which are destructive and takes away from non-invasive monitoring.

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Furthermore, interpretation of these markers requires a thorough understanding of complex interactions between cellular activities and the mechanical environment of the fracture site[96]. These markers are also used in BOC models, with the key advantages of a) the presence of a controlled environment and b) continuous monitoring.

X-Rays and Computed Tomography (CT)

X-rays and CT scanning are traditional imaging methods of monitoring fracture healing. They provide intermittent assessments, leaving gaps in understanding the dynamic nature of bone recovery, as they do not offer continuous, real-time feedback[97,98]. Dual-energy X-ray Absorptiometry (DEXA) has been used to non-invasively monitor bone regeneration due to its ability to measure bone mineral density (BMD) and assess mechanical properties[99,100] (Figure 2B). Unlike traditional radiographic methods, DEXA has been used to detect early changes in bone mineral content during healing[101,102], and for longitudinal monitoring [103,104]. These methods mainly focus on assessing the structural integrity of bone and overlook the quality and functionality of the regenerated tissue. This limitation is noteworthy in complex fractures that involve joint surfaces or multiple fragments, where structural assessments alone is insufficient[105]. High-resolution x-rays has been used to study microstructural changes in cortical and trabecular remodelling[106,107]. The use of ionising radiation in these procedures is concerning, especially where repeated imaging is required, subjecting the patient to cumulative exposure and discomfort[17].

To overcome these limitations, more imaging techniques that offer non-invasive, and real-time monitoring have been developed, however with minimal continuous monitoring.

Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT)

PET and SPECT imaging techniques assess metabolic activity, vascularisation, and cellular responses in regenerating bone (Figure 2D). PET imaging has proven effective in evaluating bone turnover and mineralisation, particularly in the early stages of regeneration, with the aid of radiotracers such as [^18F]-fluoride[18,91]. SPECT imaging has been used for assessing localised bone repair, with the use of tracers such as technetium-99m-labeled bisphosphonates[17,108]. PET and SPECT has been integrated with biomaterials and therapeutic agents to monitor their effects on bone healing. For instance, PET imaging has been used to test the efficacy of osteogenesis promoter drug delivery systems[109,110]. Hybrid PET/CT and SPECT/CT systems provide combined anatomical and functional imaging, for localisation and characterisation of bone regeneration processes[16,111,112]. These techniques have also been utilised in preclinical models to study the integration and vascularisation of engineered bone scaffolds[15], however they are limited by radiation exposure, high costs and lower spatial resolution.

Magnetic Resonance Imaging (MRI) and Quantitative Ultrasound (QUS)

Advanced imaging techniques such as MRI and QUS address some limitations of X-ray and CT methods. They are non-invasive, free from ionising radiation and are considered safe for longitudinal measurements. MRI provides information on soft tissue and bone marrow changes and assesses the overall tissue environment, although it may not fully capture the mechanical stability of the healing bone[17,113] (Figure 2E). Ultrasound, on the other hand, offers an alternative that is useful for monitoring bone callus formation and identifying early signs of complications, such as infections or delayed union[17,114,115] (Figure 2C). While these techniques

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offer a more holistic view of the healing process, MRI is limited by high costs, lengthy acquisition times and QUS face challenges due to precision errors and limited specificity caused by chemical alterations[14].

Traditional imaging modalities such as X-rays, CT, PET, SPECT, MRI, and QUS have not yet been integrated into BOC platforms. Their application in bone regeneration is presently confined to preclinical animal studies or patient imaging. For instance, DEXA and high-resolution micro-CT have been used to monitor mineral density and microstructural remodelling in-vivo[97–103,105–107,116]. PET and SPECT have shed light into vascularisation and drug efficacy using radiotracers such as [^{18}F]-fluoride or technetium-99m[108–112]. Similarly, MRI and ultrasound methods have been used to assess bone marrow, cartilage, and callus formation in fracture repair models[113–115]. While these methods are clinically relevant, their reliance on ionising radiation, high cost, or limited specificity, along with the absence of integration into microfluidic bone models suggests that their utility in BOCs remains extrapolated rather than demonstrated.

Figure 2. Examples of Current Bone Regeneration Monitoring Methods
(A) Histological cut showing details of lamellar bone concentrically organized and woven bone mixed with cartilage and calcified cartilage tissues (HE). Reproduced from reference [93] with permission from Springer Nature, copyright 2008.
(B) Total hip replacement arthroplasty (THRA) post operation evaluation example using dual energy X-ray absorptiometry. bone mineral density (BMD); bone mineral content (BMC). Reproduced from reference [103] with permission from Elsevier, copyright 2018.
(C) Ultrasonographic examination of the rabbit skull and image analysis.
A Defect location and ultrasound probe positioning during examination. B Schematic drawing (left) and corresponding ultrasound image (right) of an empty defect 24 h post surgery. C Schematic drawing (left) and ultrasound image (right) of a polycaprolactone collagen II/chitosan (PCL Coll I/CS) scaffold at 12 weeks post surgery with evident new bone formation. A defined region of interest (ROI) (red square) was used to quantify the tissue formation within the defect zone and both parietal bone ends are marked in green Reproduced from reference [16] with permission from De Gruyter, copyright 2021.
(D) A multimodality scanner in which the CT is integrated with PET and SPECT systems (Inveon System Siemens Medical Solutions, Knoxville, TN, USA). This versatile system allows unified PET, SPECT and CT data acquisition. The CT system has an automated zoom control which allows the operator to adjust the field of view and magnification Reproduced from reference [113] with permission from Springer Nature, copyright 2011.
(E) MRI images of a rat (Wistar) and mouse (C57BL/6) knee joint. (A) 3D spin echo MR image (117 × 114 × 144 μm) of a rat knee ex vivo displaying the anatomical landmarks of the articular joint: a = femur condyle, b = tibia, c = patella, d = patellar ligament, e = meniscus, f = articular cartilage and g = intrapatellar fat pad. (B) Histological image of the knee joint. The MR images provided an excellent visualisation of the rat knee anatomy, with detailed observations on the subchondral bone and in the articular synovial space. (a, b, c, d) Sequential fast spin echo multi-slices images (axial views from proximal to palmar) from the proximal region of the mouse knee (512 × 512 μm). The MR images displayed the bones of the area of knee joint (1 = patella; 2 = femur; 3, 3' = femur condyles), providing good views of the subpatellar region and the synovial cavity (see arrows). Images acquired in a 9.4-T Varian scanner (Varian, Inc., Oxford, UK) with 100 G/cm gradient coils and a Rapid bird-

cage radiofrequency (RF) coil. Reproduced from reference [113] with permission from Springer Nature, copyright 2011.

4. Emerging Methods: Photonics-Based Evaluation of Bone Healing

Longitudinal data is essential for a comprehensive understanding of bone regeneration, which helps optimise scaffold properties and reduce costs associated with animal studies and clinical trials evaluating grafts/implants. Bone healing occurs through two main pathways: primary healing, involving direct cortical restoration, and secondary healing, which progresses through inflammation, proliferation, and remodelling stages[117]. Inflammation triggers mesenchymal stem cell recruitment and differentiation, initiating callus formation, while the proliferative phase involves angiogenesis and woven bone formation through intramembranous or endochondral ossification. Advanced continuous monitoring tools allow for longitudinal data acquisition by real-time observation of these dynamic healing events for early detection. Key optical modalities are summarised in Table 1, categorised by their application stage and imaging performance (resolution, penetration depth, advantages, and limitations), with representative multimodal examples shown in Figure 3.

Table 3. Emerging Optical Methods for Monitoring Graft Optimisation

Optical Technique	Principle	Application in Bone Healing	Resolution	Penetration Depth	Spectral Range	Label-Free (yes/No)	Key Advantages	Limitations & BOC Integration Status
Live-Cell Imaging (Bright-field, Phase-Contrast, Digital Holography)	Detection of transmitted light intensity and phase shifts to visualise transparent cellular structures without staining	Cell viability, proliferation, and early extracellular matrix formation	Diffraction-limited lateral ~0.2 µm (optical microscope limit); axial ~1 µm	Shallow (~50–200 µm effective) – requires thin samples	Visible (400-700 nm)	Yes (cells imaged by intrinsic contrast).	Simple, provides morphological information comparable to histology.	Limited depth; lacks molecular specificity, BOC Integration: Demonstrated [13,118]
Fluorescence Imaging (Confocal, Multiphoton Microscopy)	Detection of light emitted from fluorophores after excitation at a specific	Visualisation of specific cells and molecular marker	Lateral ~0.2–0.3 µm (diffraction-limited); axial ~0.5–1	Confocal: up to ~100–200 µm in scattering tissue.	UV-Vis-NIR (200-900nm)	No (requires labels/probes; auto	High sensitivity and specificity; enables molecular and cellular	Photobleaching, phototoxicity, limited penetration in scattering media. BOC Integration:

Optical Technique	Principle	Application in Bone Healing	Resolution	Penetration Depth	Spectral Range	Label-Free (yes/No)	Key Advantages	Limitations & BOC Integration Status
	wavelength.	s (cell viability, proliferation, osteoblast differentiation), mineral deposition.	µm (multi-photon has slightly poor resolution for longer excitation wavelength)	Multiphoton: ~500 µm		fluorescence can be exploited in some cases)	tracking. Allows 3D imaging via optical sectioning.	Demonstrated [118,119]
Second Harmonic Generation (SHG)	Nonlinear process where two photons two photons combine to emit a photon of twice the energy; specific to non-centrosymmetric structures.	Collagen fiber (Type I/II) deposition, organisation, alignment, and remodeling.	lateral ~0.3 µm; axial ~1 µm (diffraction-limited by the NIR excitation).	~100-500 µm	Excitation: 700–900 nm IR; collected at 350–450 nm	Yes (SHG signal arises intrinsically from collagen)	Highly specific visualisation of collagen architecture, no photobleaching.	Only detects non-centrosymmetric structures (e.g., collagen, myosin); limited penetration. BOC Integration: Extrapolated from tissue models[120]
Raman Spectroscopy (Microscopy, Spatially Offset Raman Spectroscopy (SORS))	Inelastic scattering of photons, providing a chemical fingerprint based on molecular vibrations.	Mineral-to-matrix ratio, hydroxyapatite crystallinity, collagen quality, chemical	Lateral ~1 µm; axial ~2-5µm	Spontaneous Raman in tissue <100 µm SORS ~4–10 mm	Excitation: 532, 633, 785, 830 nm	Yes (intrinsic molecular vibrational contrast)	High chemical specificity; semiquantitative molecular analysis.	Weak signal, slow acquisition, autofluorescence background. BOC Integration: Extrapolated from graft/scaffold models[121–123].

Optical Technique	Principle	Application in Bone Healing	Resolution	Penetration Depth	Spectral Range	Label-Free (yes/No)	Key Advantages	Limitations & BOC Integration Status
		composition.						
Optical Coherence Tomography (OCT)	Low-coherence interferometry measuring backscattered light to create cross-sectional images.	Scaffold integration, bone microarchitecture, tissue morphology, vascular network and mineralised callus formation.	5-15 µm (lateral and axial)	0.5-2 mm >2 mm (with spatial offset between source and detector)	800-1300 nm (operates where tissue scattering and absorption are minimal, for deeper optical penetration)	Yes (based on intrinsic backscatter and refractive index differences)	Rapid 3D structural imaging at high resolution; low-power light.	Limited penetration and contrast in heavily mineralised bone; low molecular specificity. BOC Integration: Extrapolated from scaffold models[124]
Near-Infrared Spectroscopy (NIRS)/ Diffuse Optical Spectroscopy (DCS)	Measures absorption of light by chromophores (e.g., hemoglobin, water, lipids) to assess tissue composition and oxygenation.	Tissue oxygenation (StO_2), total hemoglobin, water/fat/collagen content.	Low; effectively sampling a bulk volume (~cm^3)	10-30 mm (depending on source detector separation)	NIR window (~650 - 950nm) next generation extended to 1300 nm	Yes (relies on endogenous chromophores)	Deep tissue penetration; functional monitoring of blood oxygenation.	Low spatial resolution; provides bulk measurements. BOC Integration: Extrapolated from in-vivo models[125,119].
Diffuse Correlation	Analyses temporal fluctuation	Microvascular blood	Low in 10s of mm (if	~10–30 mm (depend	750-850 nm	Yes (senses	Direct, continuous	Low spatial resolution; susceptible to

Optical Technique	Principle	Application in Bone Healing	Resolution	Penetration Depth	Spectral Range	Label-Free (yes/No)	Key Advantages	Limitations & BOC Integration Status
Spectroscopy (DCS)	s of scattered light (rapid speckle intensity) to measure red blood cell movement and infer blood flow.	flow and perfusion changes during inflammation and repair.	using multiple positions/tomography, spatial resolution can be ~5–10 mm)	ing on source detector separation)		intrinsic motion of blood cells)	measurement of blood flow index.	motion artifacts. BOC Integration: Not yet demonstrated; extrapolated from animal models[126,127].
Photoacoustic Imaging (PAI)	Combines optical excitation and ultrasonic detection. Absorbed light generates thermoelastic expansion, creating detectable sound waves.	Vascular geometry, oxygen saturation (sO_2), total hemoglobin, deep tissue structures.	lateral ~5 µm (based on depth) axial ~15–50 µm (set by ultrasound bandwidth)	30-50 mm at the expense of resolution	650-1300 nm	Yes (for blood and other endogenous absorbers)	Imaging deeper than purely optical methods; combines structural and functional imaging.	Requires acoustic coupling, complex instrumentation. BOC Integration: Extrapolated from in-vivo models[128].

Live Cell Imaging

Bright field imaging is the most common basis for 2D imaging and observing the morphology of samples. This technique has been successfully implemented to monitor cell survival and cellular proliferation. It can be further improved by incorporating phase-contrast microscopy, which enhances visualisation of transparent samples for feature quantification. For 3D imaging, differential interference contrast, holographic and confocal microscopy are useful techniques[13,118]. When combined with AI/ML, these methods are useful for studying early-stage organoid morphology and growth and further facilitate non-invasive, label-free imaging.

Fluorescence Imaging

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Fluorescence imaging provides non-invasive high-resolution, real-time monitoring of cellular activities, mineral deposition, and ECM formation[118,119] during bone regeneration. Fluorescently labelled markers help visualise indicators of bone regeneration such as osteoblast differentiation and hydroxyapatite formation[129,130], which allows continuous monitoring of osteogenic processes for longitudinal evaluation[2,131,132]. Advanced fluorescent dyes and nanoparticles complement conventional fluorescence imaging for more accurate monitoring[129,133]. Quantum dots and near-infrared fluorescent probes offer higher photostability, signal intensity, and deeper tissue penetration, deeming them better suited for long-term monitoring in complex systems[44,134]. Multiphoton fluorescence microscopy, for instance, provides high-resolution information on collagen fiber alignment and its role in osteoid formation, and has allowed real-time monitoring of mechanical stimuli and biochemical factors on matrix deposition and organisation[44,132]. Despite progress in fluorescence imaging, challenges such as photobleaching and tissue autofluorescence continue to be limitations. Fluorescence lifetime imaging (FLIM) and dual-emission probes with improved contrast and signal reliability are currently being developed to overcome these issues[135,136].

Optical Coherence Tomography (OCT)

OCT is a non-invasive imaging technique that provides micrometre-scale, real-time visualisation of tissue architecture, useful for assessing bone regeneration structurally and functionally (Figure 3E). OCT operates by measuring the echo time delay and intensity of backscattered light. This is useful for studying bone health and disease through observations of structural changes and cellular interactions in bone tissue[124,137,138]. OCT has been utilised to evaluate the integration of biomimetic scaffolds and their effects on osteogenesis, scaffold-induced changes in bone microarchitecture and vascularisation[139,140], while high-resolution OCT imaging has been used for monitoring scaffold degradation and mineral deposition[141,142]. With advancements in OCT-based angiography, combined with spatially offset, visualisation of deeper newly formed vascular networks within engineered bone constructs is possible, which helps assess successful regeneration[18]. The utility of polarisation-sensitive OCT in characterising bone matrix alignment and anisotropy during healing should also be noted, as it is helpful in assessing the mechanical stability of regenerated tissue[143].

Raman Spectroscopy (RS)

Raman spectroscopy is capable of non-invasive, high-resolution molecular analysis through inelastic light scattering. It quantifies mineral-to-matrix ratios and collagen cross-linking by measuring biochemical and structural properties of bone, such as bone mineral composition and matrix organisation during healing, and spatially resolves changes in hydroxyapatite crystallinity and carbonate substitution[122,144–147]. In the context of scaffold-based bone regeneration, Raman spectroscopy evaluates scaffold mineralisation and the integration of bone graft materials. For example, in-vivo Raman imaging has been used to monitor mineral deposition within bioengineered constructs[121,148]. It has also been used to monitor therapeutic interventions effects such as laser stimulation and pharmacological treatments on bone regeneration[149,150], and to detect *Staphylococcus epidermidis* infections in human bone grafts[123]. Advanced Raman modalities such as spatially offset Raman spectroscopy (SORS) and coherent anti-Stokes Raman scattering (CARS), have improved the ability to monitor bone regeneration at deeper tissue levels with increased sensitivity[151,152]. Recently, SORS has become a notable technique for non-invasive, in-vivo transcutaneous bone quality assessment[153]. Furthermore, technical developments in portable Raman systems has facilitated

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clinical translation by allowing bedside monitoring of bone healing[154]. However, challenges such as complex data analysis and the need for advanced miniaturised systems persist. Integration of Raman spectroscopy with other imaging techniques such as OCT and photoacoustic imaging, has the potential to comprehensively monitoring of bone health[155,156] (Figure 3E).

Near-Infrared Spectroscopy (NIRS)

NIRS measures bone regeneration non-invasively by measuring tissue oxygenation, vascularisation, bone composition changes, real-time hemodynamic responses and metabolic activity. For instance, NIRS has been used to monitor oxygen saturation and blood volume during fracture repair which are key indicators of successful vascularisation and regeneration[157,158]. In addition, by interacting with chemical bonds, 700–2500 nm range NIRS detects in-vivo variations in collagen, water, mineral, and fat content, which indicate bone quality and inform surgical planning[159]. Advances in NIRS technology, including biocompatible sensors and fiber-optic probes, have improved it's precision, hence application in tissue-engineered constructs[160,161]. Recent studies have demonstrated use of NIRS for evaluating material-tissue interactions and osteogenesis during in-vivo analysis of bone regeneration by incorporating NIRS with hydrogels and biomimetic scaffolds[125]. When combined with other imaging methods, NIRS contributes to comprehensive analysis by adding functional data to structural and biochemical information[162,163].

Diffuse Correlation Spectroscopy (DCS)

DCS non-invasively measures blood flow and microvascular changes associated with bone regeneration by analysing temporal fluctuations in scattered near-infrared light caused by the movement of red blood cells[126,127]. Advancements in DCS technology have led preclinical studies that measure localised blood flow in small animal models. In addition, Diffuse Correlation Tomography (DCT), which accounts for tissue heterogeneity and geometry, provides 3D reconstruction of blood flow[164] (Figure 3C). When combined with spatial frequency domain imaging, DCT has been used to investigate how factors such as treatment and age influence blood flow during bone repair. Preliminary findings suggest that this multimodal approach offers promising potential for improving predictions of bone healing outcomes[164].

Second Harmonic Generation (SHG)

SHG is a nonlinear optical technique where two photons combine to generate a single high energy photon with half the wavelength, providing structural information. SHG imaging provides non-invasive, label-free, high-resolution visualisation of non-centrosymmetric structures such as collagen, a key component of the bone extracellular matrix (Figure 3B). For instance, SHG was used to assess collagen remodelling, hence bone quality and its relation to mineral deposition during bone repair[165,166]. SHG imaging has also been applied to quantify structural changes in intervertebral discs and bone tissues[167,168]. Recent advancements have introduced a dual-liquid-crystal-based polarisation-resolved SHG approach for voltage-controlled polarisation modulation without the need for mechanical rotation. This technique reliably differentiates between collagen types I and II in pathological bone samples which could be used to study real-time structural changes that occur during bone fracture healing[169].

Photoacoustic Imaging (PAI)

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PAI combines optical and ultrasonic imaging to non-invasively monitor bone regeneration through high-resolution, label-free imaging of bone structures, vasculature and oxygenation[128,170] (Figure 3A). Recent advancements in optical-resolution photoacoustic microscopy have enabled VEGF-induced angiogenesis imaging, which is a key aspect of bone regeneration[171]. Functionalised nanomaterials such as gold nanorods and other contrast agents have also improved PAI's sensitivity in assessing mineralisation and osteogenesis[54,171]. In addition, PAI's ability to quantify changes in vascular and metabolic activity has been useful in evaluating bone healing treatments[172]. Integration of PAI into preclinical and clinical procedures has the potential for real-time monitoring and development of patient-specific treatments[173,174].

Real-time imaging improves bone health monitoring through continuous, non-invasive evaluation and provide a comprehensive understanding into bone regeneration, disease progression, and the effects of treatments. Techniques such as OCT provides high-resolution imaging of bone microarchitecture and healing progress, while fluorescence imaging with the aid of advanced fluorescent dyes and near-infrared probes monitors osteoblast activity and mineralisation[129,138]. Label-free monitoring of molecular changes during bone matrix formation, such as collagen cross-linking and hydroxyapatite formation is achievable via Raman spectroscopy. Vascularisation and oxygenation is visualised using PAI, combining optical and acoustic modalities for deeper tissue penetration[128,147]. In addition, DCS/DCT and NIRS have also been applied for real-time monitoring of hemodynamic changes, such as blood flow and oxygenation during healing[163,175]. The advances made through these emerging optical techniques, represents movement towards precision medicine that would facilitate better patient outcomes.

Several emerging optical techniques have begun to be incorporated directly into BOCs or closely related OOC systems. Live cell imaging and fluorescence microscopy have been demonstrated in bone-on-chip systems, allowing continuous monitoring of osteogenic differentiation and scaffold interactions[42]. However, most advanced biophotonics methods remain at the stage of extrapolation from bone tissue or scaffold models. OCT has been applied to evaluate biomimetic scaffold integration and mineralisation in engineered bone constructs[138–141], and Raman spectroscopy has monitored scaffold mineralisation and bone healing both in vivo and in engineered grafts[121,123,144–151,154–156,168], though not yet within BOCs. Likewise, NIRS has been used to assess oxygenation and collagen/mineral content in bone repair[116,125,158,160–162,176,177], and DCS has quantified blood flow in small animal fracture models[127,159,175], without BOC application to date. Other nonlinear and hybrid modalities, including SHG for collagen remodelling[157,165–167,178,179] and PAI for angiogenesis and vascular activity[128,169–173], have shown promise in bone and vascularised scaffolds, but not yet in chip-based bone models. Thus, among emerging optical tools, only live cell and fluorescence imaging are demonstrated in BOCs, while OCT, Raman, NIRS, DCS, SHG, and PAI remain extrapolated from related tissue or scaffold models.

Figure 3. Examples of Emerging Bone Regeneration Monitoring Methods
(A) Differentiation between cancellous and cortical bone through the use of photoacoustic imaging inside the vertebrae of a human cadaver. (a) Examples of photoacoustic signals generated when the tip of the optical fiber is touching cortical bone (i.e., a medial breach) and a cancellous core. Each row shows the CT axial slice and corresponding coherence-based ultrasound image coregistered with a delay-and-sum photoacoustic image. There is a compact

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photoacoustic pattern when the optical fiber tip is touching cortical bone and a diffuse pattern when it is surrounded by cancellous bone. (b) Areas of −6 dB contours around the center of photoacoustic targets from cortical and cancellous bone using delay-and-sum beamforming. Abbreviations: CT, computed tomography; HU, Hounsfield unit. Reproduced from reference [128] with permission from Annual Reviews, copyright 2023.

(B) Overview of polarisation-resolved second harmonic generation (pSHG) microscopy. (a) A single SHG image of type I collagen fibres in the non-mineralising (NM) region of a turkey leg tendon (TLT). (c) The SHG image from a, overlaid with coloured arrows. The direction of each coloured arrow indicates the dominant direction around which the SHG harmonophores were aligned ($\boldsymbol{\varphi}2$), and the colour of each arrow depicts the degree of organization ($\boldsymbol{I}2$). For clarity, arrows are only printed for every 10th pixel. Scale bar = 50 μm. Reproduced from reference [165] with permission from The Royal Society, copyright 2024.

(C) DCT optical measurements. An example of the three-dimensional relative blood flow (rBF) changes in a mouse with an allograft (Mouse 10). Each row shows the z-slice of the three-dimensional rBF distribution at each week and each column shows the temporal changes for a specific z-slice. The borders of the bones and graft are outlined with white and red lines, respectively. Reproduced from reference [175] with permission from PLOS, copyright 2018.

(D) Schematic overview of a biomimetic bone-on-a-chip platform combined with AI-based image analysis for high-throughput drug testing. (a) Illustration of 3D osteon niche of bone in vivo. (b) The configurational mimicking of bone in the biomimetic bone-on-a-chip platform. Immature osteocytes were embedded in collagen and osteoblast-derived decellularised extracellular matrix (OB-dECM) composite rat tail collagen type I and OB-dECM (Col/OB-dECM) within a chip, and preosteoblasts were cocultured in a region around the chip. Both cells were differentiated and matured to bone in bone-on-a-chips built in a well plate. (c) Illustration of osteoporosis drug testing based on this platform and image data analysis using deep learning algorithms. 3D, three-dimensional; AI, artificial intelligence; OB-dECM, osteoblast-derived decellularised extracellular matrix. Reproduced from reference [60] with permission from WILEY, copyright 2022.

(E) Schematic of a multimodal RS and Spectral-domain optical coherence tomography (SD-OCT) system with separate detection sub-systems, combined through a shared sample arm. This example shows spectral channels separated by dichroic filters (DF), and detected by dedicated spectrometers with collimating lens (CL), diffraction grating (DG), and charge-coupled device (CCD). Reproduced from reference [180] with permission from WILEY, copyright 2022.

5. Challenges and Future Directions

The integration of BOC models with biophotonics-based monitoring techniques has advanced in-vitro bone regeneration studies, yet several critical challenges continue to hinder their clinical translation (Figure 4C). While these platforms have demonstrated promise, their impact will be limited unless fundamental hurdles from reproducibility to scalability are addressed in an effective, timely manner. Below, guided by literature, we offer our opinion on what are considered key challenges associated with BOCs and imaging modalities, and steps that could be taken to successfully overcome these. A visual representation of some of such challenges and opportunities are given in Figure 4.

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Physiological Relevance and Systemic Integration

In-vivo, bone regeneration is driven by mechanobiological stimuli and dynamic blood supply, which are not recapitulated with sufficient fidelity by BOCs, which is a critical gap in mimicking osteogenesis[3,181]. BOCs effectively replicate cellular and molecular bone environments, and current vascularisation methods such as endothelialised microchannels and perfusable scaffolds have improved nutrient exchange[182] (Table 1, Figure 1B).

The integration of immune system components into BOCs is also in its early stages, despite their crucial role in inflammation-mediated bone healing[12]. Therefore, to successfully address the challenge of physiological relevance of BOCs, they would need to be designed as part of a systemic, interconnected physiological network, such as multi-organ-on-a-chip and human-on-chip (HOC) platforms, rather than BOCs that mimic bone as an isolated tissue. A shift towards more biologically complex BOC models is necessary to make accurate prediction of human responses.

Such development could be supported by advanced biophotonics-based monitoring tools (Table 3, Figure 3), which could validate fidelity of these systems against in-vivo benchmarks and guide refinement. They could also confirm effective mimicry of complex, systemic interactions in highly complex systems such as HOCs within clinical settings providing a compelling rationale for improving real-time imaging techniques.

Monitoring Gaps and the Need for Advanced Imaging

The need for real-time, non-invasive monitoring in BOCs is a motivating factor for advancements in biophotonics-based imaging, which arises from the need to detect implant failure, delayed healing, or infection at early stages. These factors, if overlooked, could lead to long-term complications and increased healthcare costs. The ability to longitudinally monitor bone repair, non-invasively or without ionising radiation, is increasingly viewed as essential in regenerative medicine.

Yet, emerging techniques (Table 3) are limited by lack of adequate penetration depth, resolution and standardisation. For instance, Raman spectroscopy, OCT, and SHG imaging provide high-resolution data on bone matrix mineralisation, however, are limited in visualising deep tissue structures[10]. Multimodal imaging (Figure 3E) approaches that integrate optical, X-ray based, and acoustic techniques could be a potential solution, however are limited by challenges in data fusion and interpretation[43]. These limitations, combined with variability in device performance and a lack of standardised calibration protocols, can result in inconsistent data, which affects reliability.

Diffuse optical imaging methods, such as NIRS and DCS/DCT, have demonstrated use in vascularisation and metabolic activity assessments, however, are limited in their sensitivity and spatial resolution for accurate, quantitative analyses[28]. Therefore, further refinement is required to realise the full potential of these imaging tools within BOC platforms.

Lessons from optical integration in other microfluidic tissue models

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Experience from other microfluidic tissue models could be useful for illustrating how optics can be effectively integrated for non-invasive monitoring and may guide the refinement of BOCs:

- **Barrier and transport (gut, lung) systems:** Optical sensors, often coupled with Mitral transcatheter edge-to-edge repair (TEER) or live fluorescence imaging, have been used to monitor epithelial barrier integrity and transport dynamics, demonstrating the value of label-free, continuous readouts[183].
- **Vascular systems:** Integrated fluorescence and OCT methods have provided real-time assessment of vascular permeability and angiogenesis, emphasising the need to match optical sensing modalities to physiological processes[184].
- **Cardiac tissues:** Optical mapping of calcium flux and contractility has provided information on dynamic behavior not accessible through electrical sensors alone, demonstrating the complementarity of multimodal approaches[185].
- **Cross-cutting lessons:** Across these models, success relies on (a) continuous, on-chip monitoring to enhance the applicability of the data collected; (b) combining optical with electrical/chemical modalities to reduce ambiguity; and (c) robust packaging and alignment strategies to ensure reproducibility and sterility[186].

These examples demonstrate that integrating optics into microfluidic platforms is most impactful when tailored to tissue-specific functional readouts while ensuring system robustness, a principle equally relevant for BOCs.

Lack of Standardisation and Regulatory Readiness

Standardisation is an unmet need in BOC development (Figure 4A). Variations in device fabrication, microfluidic design, and biomaterials create inconsistencies across studies, affecting reproducibility and making it difficult to compare results or translate findings into clinically applicable formats[90]. In the absence of standardised fabrication and operational methods, BOCs risk becoming niche academic tools rather than useful biomedical solutions (Figure 4B).

Economic and regulatory barriers delay the widespread adoption of BOCs despite technological advancements. Microfabrication, advanced biomaterials, and imaging devices are costly and resource-intensive, creating a financial barrier that limits these systems to well-funded institutions[187]. Even if the cost burden could be reduced, regulatory ambiguity is yet to be addressed, as existing biomedical device regulations are not well adapted to microfluidic models. And, as regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) require consistent and reproducible data for device approval[188] this creates challenges in commercialisation and clinical integration[189].

However, the regulatory landscape is evolving. The acceptance of the FDA Modernization Act 2.0 in 2022 indicated a pivotal shift that allow drug sponsors to use data from alternative methods, including OOCs, in place of animal testing to demonstrate safety and efficacy. To support this the FDA and the National Institutes of Health (NIH) actively facilitate and engage in programs such as the Translational Centers for Microphysiological Systems (TraCe MPS) who's aim is to validate and qualify tissue chips as official drug development tools[190]. Similarly, the EMA has established expert communities to guide the integration of New Approach Methodologies (NAMs), including OOCs, into their regulatory frameworks[191].

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International efforts in standardisation are gaining momentum. The European Committee for Standardisation (CEN) and the European Committee for Electrotechnical Standardisation (CENELEC); CEN-CENELEC Focus Group on Organ-on-Chip, supported by the European Organ-on-Chip Society (EUROoCS), recently published a comprehensive roadmap outlining key areas for standardisation, including terminology, minimum reporting requirements for cells and biomaterials, and technical specifications for device hardware and data management[192].This work is intended to inform future standards developed by the International Organization for Standardisation (ISO), particularly within its technical committee on biotechnology (e.g., ISO/TC 276). Such standards, along with existing ones such as the ISO 10993 ("Biological evaluation of medical devices"), are essential for ensuring reliability and interoperability of BOC platforms, leading to their commercialisation and clinical integration[189]. Therefore, a combined effort from academics, industry, and regulatory bodies would be required to navigate these challenges and establish clear pathways for BOC validation and approval.

Opportunities and Outlook

In our opinion, the future of BOC technology depends on its ability to successfully overcome current limitations by integrating tissue engineering, biophotonics, AI/ML approaches and other complementary techniques that would help bridge the bench-to-bedside gap in bone regeneration therapies (Box 2, Figure 3, 4A).

A priority is optimisation of biophotonics-based imaging within BOCs for continuous, longitudinal assessment of osteogenesis and implant integration. Future research should focus on multimodal configurations for layered information across spatial and biochemical dimensions. Though AI-driven image analysis and machine learning algorithms have demonstrated their potential in processing large amounts of imaging datasets (Figure 3D), they are equally or even more useful in predictive modelling[5]. If AI could accurately predict bone healing outcomes based on real-time imaging, BOCs could become a core feature of personalised medicine, optimising treatments for individual patients. The integration of smart biomaterials, including bioactive hydrogels and mechanically tunable scaffolds would be key to advancing physiologically relevant BOC models[3]. However, materials innovation alone may not be sufficient. The field must also develop towards multi-organ integration allowing BOCs to interact with vascular, neural, and immune components to create holistic, systemic models of bone regeneration[188].

Ultimately, the refinement of vascularised, immune-responsive, and AI-enhanced BOC platforms represents the next step in bone tissue engineering. These models must not only mimic bone biology but also address the complexities of real-world clinical applications.

Figure 4. Challenges and Requirements/Future Directions of BOC Technology
(A) Emerging technologies including biofabrication, multimodal imaging, and AI modelling have the potential to expand the capabilities of OOC platforms, thus accelerating model validation and facilitating regulatory acceptance to ultimately lead to standardized tools adopted by main stakeholders in the drug development pipeline. Reproduced from reference [193] with permission from Frontiers Media, copyright 2024.
(B) Building blocks to standard integration of AI-based models into OOC platforms. 1) Problem definition. 2) Optimisation of OOC experiments, including OOC design, cell sources, and perfusing media. 3) Imaging data acquisition with consistent sample preparation, constant light/exposure conditions, and same magnification settings. 4) Adjust to data “fit-

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for-use" for AI-based models by performing data pre-processing techniques, data splitting, and adding metadata. 5) Training and validation of an explainable, interpretable, and unbiased AI-based model. 6) Generate new data of control and treated cells to test the previously developed model. 7) Analyse the predicted outcomes and refine the model according to suggestions from both academic and industry stakeholders. 8) Implement and disseminate the model. Reproduced from reference [193] with permission from Frontiers Media, copyright 2024.
(C) Intrinsic correlation between the process complexity of the bioengineered bone microenvironment and its translational potential. Simple and advanced strategies have been proposed to recapitulate the bone microenvironment. While the first one approach has shown an elevated translation potential, the incorporation of several elements (e.g., biomaterials, cellular and noncellular components, different approaches, technologies, and culture systems) has limited the translational potential of the second approach Reproduced from reference [194] with permission from AIP Publishing, copyright 2021.

6. Conclusion

BOC technology has advanced in-vitro bone regeneration research by replicating the complex bone healing environment (Tables 1 and 2). However, real-time, continuous, non-invasive monitoring of osteogenesis, required for advancing fundamental research and clinical translation is an ongoing challenge. Optical techniques (Table 3), such as Raman spectroscopy, SHG, OCT, and NIRS, provide high-resolution, label-free imaging of bone formation, while PAI and DCS/DCT provide deep tissue information into vascularisation and metabolic activity. Multimodal imaging and integration of AI-driven image analysis have improved automation of osteogenic monitoring within BOCs leading to individualised regenerative approaches. Biosensors and optoelectronic systems embedded within BOCs have made real-time biochemical analysis possible, providing a comprehensive assessment of bone health (Box 2).

Despite these advancements, challenges in standardising fabrication methods, reproducibility, and developing vascularised and immune-responsive models as better mimics remain. Addressing these limitations would be key to scaling BOCs for clinical applications. The authors believe that, ultimately, the convergence of BOCs with biophotonics-based monitoring represents a major step towards non-invasive, continuous, and real-time assessment of osteogenesis, that would improve preclinical bone research and its clinical translation. With further advancements, these platforms will play a pivotal role in improving clinical outcomes in bone regeneration.

Author Contributions
IJ led manuscript and figure preparation and focused on BOC systems and optical methods. RG assisted with manuscript preparation and focused on optical methods. SAE provided feedback on the review.

Conflicts of Interest
The authors declare no conflicts of interest.

Data Availability Statement
No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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Integration of Biophotonics with Bone-on-Chip Technology for Continuous, Non-Invasive Monitoring of Bone Regeneration

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Imanda Jayawardena[1*], Stefan Andersson-Engels[1,2] and Rekha Gautam[1]

**imanda.jayawardena@tyndall.ie*
1. Tyndall National Institute, Lee Maltings Complex, Dyke Parade, Cork, Co Cork T12 R5CP, Ireland.
2. Department of Physics, University College Cork, Cork, Co Cork T12 K8AF, Ireland.

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.