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# Modeling the effects of radiation on the bone tumor microenvironment: opportunities for exploring combination therapies in microphysiologic systems

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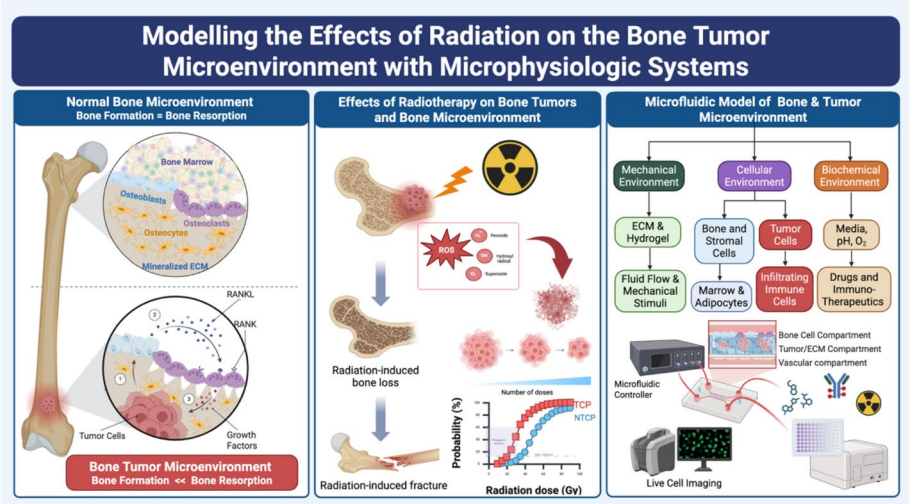
## Abstract

Primary bone tumors and bone metastases represent significant challenges in oncology. Radiotherapy is an important adjuvant treatment for several primary bone and musculoskeletal tumors, as well as for palliative care for metastatic bone lesions. While effective in these applications, patients receiving skeletal radiation face a lifelong risk of fragility fracture at the irradiated sites, among other complications. Damage to bone could be reduced by development of tumor-selective radiosensitizers that would enhance the efficacy of radiotherapy, resulting in reducing the radiation dose delivered to the normal tissues. The creation of bone-selective radioprotection and radio-mitigant strategies that could respectively reduce the magnitude of off-target damage and stimulate functional recovery of the healthy bone microenvironment are warranted. Key barriers to progress in this field include the paucity and inconsistency of data on the skeletal effects of radiotherapy, low throughput and high cost of animal models, reproducibility challenges with in vitro experiments, and poor translational relevance of these models, which may not accurately replicate the human bone-tumor microenvironment. Microphysiological systems (MPS) will accelerate progress in this field by enabling rapid and cost-effective investigation while recapitulating the complexity of the bone-tumor microenvironment to more accurately model the collective response to therapy. Here, we summarize the current knowledge on the transient and long-lasting impacts of radiotherapy and explore opportunities for MPS to streamline and expand our knowledge base. We critically evaluate contemporary model systems, including MPS, and offer suggestions for how these systems can be used to efficiently model the intersection of skeletal radiobiology and bone cancer, and accelerate development of combination therapies.

**Keywords:** Bone-tumor microenvironment, Radiation, Combination therapy, Microphysiological systems

**Graphical Abstract**




## Introduction

Patients with primary and metastatic bone tumors, whether malignant or benign, can present with significant pain, paraneoplastic symptoms associated with locally altered bone turnover, predisposition to pathologic fracture, and poor disease outcomes [1]. In the USA, there is estimated to be 62,000 people living with primary bone tumors, including those with osteosarcoma, Ewing sarcoma, and chordoma. Approximately 100,000 people develop bone metastases every year, which for many cancers can signify a shift in treatment objectives from curative to palliative. Primary bone cancers primarily affect children, adolescents, and young adults, while benign bone tumors, skeletal metastases, undifferentiated pleiomorphic sarcomas, myeloma, and secondary bone tumors are more prevalent in older adults. Despite advances in chemotherapy and other systemic therapies, surgery and adjuvant radiation are the primary means of obtaining local control of most bone tumors [2–4].

Despite being a valuable method to obtain local tumor control, radiation can damage the surrounding healthy tissues and can adversely affect quality of life for the patient [5]. The off-target effects of bone irradiation include increased risks for limb-length asymmetry and angular deformity in skeletally immature patients due to growth plate injury [6, 7], and a life-long elevated risk of fracture, which may occur in up to 42% of patients [8–12]. One of the major challenges in developing strategies to mitigate off-target toxicity is the limited knowledge of how ionizing radiation affects the bone microenvironment. The effectiveness of radiation may be improved through combination therapy approaches that exploit radiation-induced microenvironmental changes to favor tumor eradication and preservation of normal bone tissue function [13].

The bone microenvironment is populated by diverse cell populations derived from distinct mesenchymal, hematopoietic, vascular, and neural lineages, each with unique homeostatic functions and responses to radiation. Given the cellular complexity of the bone microenvironment, significant challenges exist in modeling how radiation affects these populations, particularly in the context of bone tumors. MPS are a rapidly evolving

technological approach to efficiently model complex systems, while also enhancing physiologic relevance by incorporating organotypic cells, biomimetic extracellular matrices, and application of mechanical and biophysical stimuli [14]. The recent explosion of MPS [15] has enabled rapid assessment of the microenvironmental modulating effects of radiation and of the effectiveness of combinatorial therapeutic approaches [14]. However, there are few studies in the current literature that use MPS to probe the intersection of skeletal radiobiology and orthopedic oncology. The objective of this review is to summarize the current knowledge regarding the effects of radiation on the bone microenvironment, survey the use of MPS to model skeletal biology and radiobiology, and propose opportunities to merge these fields to accelerate the development of novel combinatorial therapeutic strategies to improve treatment of radiosensitive bone tumors.

## The physiology of the bone microenvironment

### The organization of cellular populations in the bone microenvironment

Bone is a mineralized connective tissue that gives structure and support to the body, serves as a reservoir for calcium and phosphorus, participates in homeostatic regulation, and provides a supportive niche for hematopoiesis. Cortical bone provides structure and mechanical stability [16]. In large mammals, including humans, the cortical compartment of the long bones—those formed by endochondral ossification—are organized into semi-cylindrical units called osteons. At the center of which is the Haversian canal which contains a bundle of arterial, venous and lymphatic vessels, and nerves. Volkman canals run perpendicular to the osteons, connecting the neurovascular structures to the haversian canals. The cortex of flat bones—those formed by intramembranous ossification—are considerably thinner and lack osteonal organization. The cortex of flat bones is innervated and perfused by networks of endosteal and periosteal vasculature and nerves, with occasional transverse channels connecting them. It is important to note that the long bones of small mammals (rats, mice) commonly used in research also form by endochondral ossification, but their cortices lack Haversian organization and are perfused and innervated in a manner like that described for the flat bones. Within the cortical compartment, trabecular or spongy bone provides structure for the marrow compartment, contributes to the mechanical strength of the bone by internally buttressing the cortex, and facilitates dynamic regulation of systemic ion homeostasis through the coupled activity of osteocytes, osteoblasts, and osteoclasts [17]. The bone extracellular matrix is a composite material secreted by cells of the osteochondral lineage and is composed of an organic phase consisting of proteins, proteoglycans and glycosaminoglycans, and an inorganic mineral phase [18–21].

The vasculature of the bone serves as a scaffold for diverse functional niches with unique cell populations. Cortical bone is supplied by blood vessels coursing through the Haversian canals, as well as endosteal and periosteal networks [22]. The periosteal system is an extensive network of vessels in the outer third of the bone that run through the length of the bone shaft [23, 24]. Periosteal arteries divide into branches and enter the cortex through Volkmann's canals to supply the cortex with blood [23, 24]. The arterial network supplying the trabecular-medullary compartment [25–27] is physiologically subclassified into high-velocity capillary-like type H vessels in areas of robust bone formation, and low velocity sinusoidal type L vessels, which sustain the bone marrow and

may provide a specialized niche for pericyte-like mesenchymal osteoprogenitors and hematopoietic stem cells [28–32]. Drainage occurs through the central venous sinus, nutrient veins, periosteal veins, and emissary veins [24]. Lymphatic vessels traverse through the cortical bone and the bone marrow and support hematopoiesis and bone regeneration [24].

The major bone-specific cells are osteoblasts, osteocytes, and osteoclasts. Osteoblasts are derived from mesenchymal stromal progenitors. They reside on the bone surface where they secrete and mineralize extracellular matrix [16]. Some osteoblasts will become entombed in the matrix they secrete and evolve into terminally differentiated osteocytes [16]. Osteocytes are the most numerous cells in the bone and serve as the primary mechanosensory cells of bone tissue. While the osteocyte cell body remains within a mineralized lacuna, osteocytes extend dendritic processes that create and maintain small canals called canaliculi [33–38]. Through the canaliculi, osteocytes connect to each other via gap junctions that allow for the propagation of mechanically evoked signals and facilitate transfer of nutrients between cells and blood vessels within the bone [16, 17, 39–42]. Furthermore, osteocytes play a key role in bone mechanoadaptation by orchestrating osteoblast and osteoclast activity [43], and contribute to systemic endocrine regulation or ion homeostasis and metabolism [44–47].

Osteoclasts are multinucleated cells derived from the myeloid-macrophage lineage responsible for bone resorption [17]. In addition, the bone marrow is home to a host of immune cells and mesenchymal stromal cells including specialized marrow adipocytes, which integrate paracrine and endocrine signals through bidirectional feedback loops that modulate bone homeostasis and contribute to regulation of systemic metabolism [48]. Thus, the bone is home to diverse cellular populations that support its functional needs [43] and contribute to systemic endocrine regulation, ion homeostasis, and metabolism [44–47].

#### Bone remodeling and hematopoiesis

Bone maintains structural integrity and systemic homeostasis through tightly regulated remodeling [45, 49]. Remodeling occurs in three phases—resorption, reversal, and formation—driven by osteoclast–osteoblast coupling [17, 50]. Osteoclast precursors express receptor-activator of nuclear factor k (RANK, *TNFRSF11A*). Osteoblast lineages regulate osteoclast maturation by secreting receptor-activator of nuclear factor k ligand (RANK-L, *TNFSF11*), which stimulates osteoclast formation by inducing cytoskeletal remodeling and secretion of proteases such as cathepsin K and matrix metalloproteinases (MMPs) necessary for bone degradation [17, 51, 52]. Osteoblast-lineage cells can also restrain osteoclast formation by secreting the RANK paralog osteoprotegerin (OPG, *TNFRSF11B*), which functions as a soluble decoy receptor that neutralizes RANK-L [17, 52]. In contrast, osteoclasts recruit and activate osteoprogenitors through activation of latent matrix bound BMP/TGFb family ligands during bone resorption [53–55], and express Notch [56] and WNT [57–59] ligands that regulate osteoblast development in a paracrine manner. Within the bone microenvironment, osteoblasts and other bone marrow stromal cells, including fibroblasts and endothelial cells, create a niche that regulates HSC populations and directs their lineage commitment via direct interactions and secretion of cytokines, while maintaining vascular access for blood and immune cells

to enter the systemic circulation. Radiation injury disrupts the delicate balance of cell activity in the bone microenvironment, setting the stage for fragility fracture and other adverse events.

## Cellular biology of the bone tumor microenvironment

The bone tumor microenvironment (TME) is a highly dynamic and multicellular ecosystem in which tumor, stromal, vascular, neural, adipocytic, and hematopoietic elements interact to drive disease progression, therapeutic resistance, and systemic complications. The presence of malignant cells in the bone microenvironment initiates a pathological deviation from homeostatic bone physiology, characterized by tumor-driven rewiring of cellular interactions, extracellular matrix (ECM) remodeling, and biochemical signaling.

### Cellular reprogramming and stromal co-option

Bone tumors exploit and rewire stromal populations such as mesenchymal stromal cells (MSCs), nerves, and adipocytes to support tumor progression and reshape the bone microenvironment. Tumors hijack MSC differentiation to disrupt bone remodeling, as seen in osteosarcoma, where TP53/RB1 loss drives IL-6, CCL5, and CXCL12 secretion that activates STAT3/NF-κB signaling to promote metastasis [60], In Ewing sarcoma, rapid tumor growth and pathologic angiogenesis induces local hypoxia and acidification of the TME, inducing cells in the osteoblast lineage to secrete factors that stimulate osteoclast-driven osteolysis [61]. Metastatic breast cancer recruits TGF-β-polarized Cancer-associated fibroblasts (CAFs) from MSCs to create immunosuppressive niches [62], while prostate cancer metastases promote aberrant osteoblast activation through endothelin-1 and BMPs, leading to sclerotic lesions [63]. Nerves are also co-opted by tumor cells in the bone microenvironment. For example, sensory nerve-derived calcitonin gene-related peptide (CGRP) drives proliferation and osteolysis in breast and prostate cancer [64]. Ewing sarcoma cells overexpress nerve growth factor (NGF), attracting TrkA+ nerve fibers that promote vascular leakiness and metastatic spread [61]. Tumor-induced acidosis sensitizes TRPV1+ nociceptors, contributing to cancer-induced bone pain and suppresses CD8+ T-cell cytotoxicity [65]. Clinical studies link perineural invasion of prostate cancer biopsies to an 11-fold increased risk of bone metastasis, underscoring the prognostic value of neural interactions [66]. Bone marrow adipocytes (BMAs), which can comprise up to 70% of marrow volume in adults, influence tumor progression by sequestering lipophilic drugs [67], enhancing β-oxidation via FABP4 under hypoxia [68], and promoting growth of metastatic prostate cancer in bone [69]. They also modulate the tumor microenvironment by secreting chemokines that recruit immunosuppressive cells [70], increasing in volume with Androgen deprivation therapy [71], activating JAK2/STAT5B signaling [70, 72], and reducing ECM stiffness by reducing integrin α5β1/FAK signaling [73].

### Immunomodulation and immune evasion

The bone TME suppresses anti-tumor immunity through context-dependent mechanisms. Myeloma upregulates PD-L1 on tumor cells and CD38-mediated adenosine production, directly inhibiting cytotoxic T-cell function [61, 70]. Osteosarcoma polarizes tumor-associated macrophages (TAMs) to an M2 phenotype via IL-10 and

TGF-β, blunting CD8+ T-cell responses [74]. Breast cancer metastases secrete CXCL12 to recruit regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), creating an immunosuppressive niche that shields disseminated tumor cells from immune surveillance [75].

#### ECM and biomechanical remodeling

Tumor-induced ECM alterations vary by malignancy. Osteolytic metastases (e.g., myeloma, breast cancer) release Cathepsin K and MMP-9, degrading collagen to liberate embedded IGF-1 and PDGF, which fuel tumor growth [76]. In contrast, osteoblastic prostate cancer lesions exhibit elevated collagen crosslinking and matrix stiffness ($\geq$30 kPa), activating integrin β3/FAK signaling to enhance invasion [77]. Primary sarcomas, such as Ewing sarcoma, express high levels of vascular endothelial growth factor (VEGF) that stimulate rapid angiogenesis, creating poorly organized networks of immature vessels that facilitate hematogenous spread [78]. These biomechanical changes impair normal bone repair mechanisms, leading to pathologic fractures and sclerotic lesions with reduced mechanical integrity.

#### Bone marrow adipocytes: metabolic architects of the TME

Bone marrow adipocytes (BMAs) constitute up to 70% of marrow volume in adults and exhibit tumor-contextual roles. Mature BMAs sequester lipophilic drugs (e.g., doxorubicin) within lipid droplets, reducing intracellular concentrations in myeloma and breast cancer cells by 30–50% [67]. Adipocyte-derived fatty acids upregulate FABP4 in tumors, enhancing β-oxidation and survival under hypoxia [68], and may contribute to growth of metastatic prostate cancer in bone [69]. In breast cancer, BMAs secrete IL-8 and CCL7, recruiting CCR2+ MDSCs that inhibit natural killer (NK) cell activity [70]. Androgen deprivation therapy can increase the volume of bone marrow adipose tissue [71], whose local production of leptin resulting in JAK2/STAT5B activation driving castration-resistant progression [70, 72]. Adipocyte-enriched niches decrease ECM stiffness (< 25 kPa), reducing integrin α5β1/FAK signaling, potentially impacting invasiveness and metastasis [73].

#### Systemic and paraneoplastic effects

Local disruption of the bone microenvironment caused by the presence of tumor cells can have systemic ramifications, such as hypercalcemia, anemia of chronic disease, and immune exhaustion, which can adversely affect patient outcomes [79]. Hypercalcemia of malignancy often results from osteolytic metastases, whose local bone destruction via tumor derived RANK-L and/or PTHrP-driven osteoclast activation, can raise serum $Ca^{2+}$ levels > 12 mg/dL, producing an array of paraneoplastic symptoms and severe bone pain [79]. Tumor-associated macrophages (TAMs) secrete hepcidin, reducing iron availability for erythropoiesis and inducing anemia via IL-6/STAT3-mediated suppression of erythroblast differentiation [80]. Other paraneoplastic syndromes affecting the bone microenvironment include ectopic adrenocorticotropic hormone (ACTH) production by small cell lung cancer metastases, causing Cushing syndrome, and VEGF/PDGF-driven hypertrophic osteoarthropathy in osteosarcoma, characterized by periosteal new bone formation at primary and metastatic sites [75].

## Modeling the bone-tumor microenvironment with microphysiologic systems

The dysregulated bone tumor microenvironment (TME) presents both challenges and therapeutic opportunities. Hypoxia-activated prodrugs (e.g., TH-302) and CXCR4 inhibitors are under investigation for primary tumors [81], while bisphosphonates and radiotherapy help manage metastatic bone disease by slowing bone destruction, but do not restore homeostasis or promote new bone formation [75]. Emerging strategies aim to exploit radiation-induced vascular permeability to enhance drug delivery and combine immunotherapy with radiotherapy to capitalize on transient immune activation. Preclinical testing of these approaches can be enhanced by using MPS to model niche-specific features such as acidosis, hypoxia, and ECM stiffness. For example, three-dimensional (3D) bioprinted osteosarcoma models incorporating BMAs and neurons show CXCL12-driven homing to RANK-L-rich niches [82], and droplet-based microfluidics assessed Ewing sarcoma spheroids under simulated shear stress [83].

MPS platforms meet this need by integrating key features such as ECM stiffness, hypoxia gradients, co-culture systems, and perfusion dynamics. These models allow high-throughput studies of radiation's effects on bone, stromal, vascular, neural, hematopoietic, and tumor compartments [84–86]. MPS models also enable dissection of radiobiological timing, identification of therapeutic windows, and testing of combination therapies that exploit transient microenvironmental shifts.

The following sections will explore how ionizing radiation alters the bone TME at the cellular and molecular levels, and how MPS technologies are advancing our understanding of vascular, neural, and adipocytic remodeling, hematopoietic suppression and recovery, and immune dynamics. We will highlight how these models accelerate development of radiosensitizers, radioprotectants, and multimodal therapies aimed at improving outcomes for patients with bone tumors.

## Ionizing radiation as a cytotoxic agent

Radiotherapy is administered to over 50% of patients with cancer, primarily for solid tumors, including bone malignancies. It plays a critical role alongside surgery and chemotherapy, particularly for local control of inoperable tumors or cases with residual disease. External-beam radiotherapy is the most common modality used for bone tumors, while brachytherapy has also been explored for bone metastases [87, 88] and bone adjacent soft tumors [89–91]. Advanced imaging technologies, intensity-modulated radiation therapy (IMRT), and stereotactic body radiation therapy (SBRT) have enhanced targeting precision, allowing sparing of surrounding healthy tissue.

While most patients with solid tumors will receive photon radiotherapy [92, 93], which will be our focus in this review, particle therapies, particularly proton and heavy-ion radiation, are becoming increasingly accessible. Unlike conventional photon or electron therapy, these novel modalities exploit the Bragg peak to deliver highly conformal doses with minimal exit radiation, offering potential advantages for tumors near critical structures or requiring high dose precision.

Radiotherapy induces tumor cell death primarily through the formation of DNA double strand breaks (DSB) [92]. While direct ionization of DNA occurs, most damage arises indirectly through radiolysis of water, generating reactive oxygen species,

including hydroxyl radicals. These initiate the DNA damage response (DDR), leading to cell cycle arrest, repair, or, if the damage is irreparable, cell death via apoptosis, mitotic catastrophe, or autophagy. Some damaged cells may enter senescence, persisting until cleared by immune-mediated mechanisms such as pyroptosis [92].

The DDR is initiated by the sensor kinases ATM and ATR, which detect DSB and single-strand lesions, respectively [94, 95]. These kinases phosphorylate downstream effectors, including Chk1 and Chk2, coordinating cell cycle check points and repair responses [96, 97]. Two major DSB repair pathways are activated. Homologous recombination (HR), a slow but high-fidelity process requiring a sister chromatid, is primarily active in S/G2 phases [98–100]. Non-homologous end joining (NHEJ) functions throughout the cell cycle and involves direct ligation of broken DNA ends, making it more error-prone [101–104]. Core components include the MRN complex (Mre11, Rad50, Nbs1), BRCA1/2 for HR [105], and DNA-PKcs, Ku70/80 [106, 107], Ligase IV, and XRCC4 [108] for NHEJ.

Cell fate decisions following irradiation are regulated at key checkpoints. ATM/ATR signaling stabilizes p53 via phosphorylation, promoting p21-mediated inhibition of Cyclin E/CDK2 to arrest cells at G1/S [94, 109–111]. At the intra-S and G2/M checkpoints, Chk1/Chk2 phosphorylation leads to degradation of Cdc25 phosphatase, halting replication or mitotic entry [94, 112–115]. Sustained G2 arrest due to unrepaired damage results in cell death, while cells with repaired DNA are released into mitosis [115]. Finally, the spindle assembly checkpoint in mitosis ensures proper chromosome segregation. Cells undergoing mitotic catastrophe display characteristic nuclear abnormalities (micronuclei, multinucleation) and typically die through delayed apoptosis or necrosis in the subsequent interphase. Unresolved damage may result in apoptosis, senescence, or mitotic catastrophe, characterized by nuclear fragmentation, micronuclei formation, and delayed cell death in subsequent cycles triggered by chromosomal imbalance.

Radiotherapy remains central to treating certain bone and musculoskeletal tumors, but its effects extend beyond cytotoxicity, impacting osteoblasts, osteoclasts, vasculature, adipocytes, neurons, and immune cells through a cascade of acute and chronic responses [70, 75, 116]. These include increased vascular permeability, immune cell recruitment, stromal activation, marrow adiposity, fibrosis, neural remodeling, and persistent immunosuppression.

Radiation-induced hypoxia and acidosis may transiently sensitize tumors but also activate pro-survival and immunosuppressive programs in stromal and immune cells [117]. Vascular injury can enhance immune infiltration and drug access but may also support metastatic niches and endothelial dysfunction [118–121]. Neural remodeling, including sensory nerve activation and neurotrophin-mediated axonogenesis, not only contributes to cancer-induced bone pain but may also facilitate tumor progression and immune evasion [64]. Irradiation-induced expansion and metabolic reprogramming of bone marrow adipocytes promote chemoresistance and disrupt hematopoietic balance [70, 122–124].

The biological effects of radiation on the bone microenvironment vary significantly depending on the radiation type [125]. Conventional X-rays deposit energy along their path, exposing both tumor and surrounding healthy tissue, which can lead to widespread stromal remodeling and hematopoietic suppression [126]. In contrast, radiotherapy with protons, neutrons, or heavier ion species exploit the Bragg peak to deposit maximal

energy at a defined depth, thereby reducing off-target effects and preserving bone marrow integrity [127] and producing complex, clustered DNA damage that is more difficult to repair [93]. While these features enhance tumor control, they may also intensify local tissue injury, especially in radiosensitive stromal compartments [128, 129]. Current evidence suggests that heavy ions may cause greater osteoblast depletion and bone fragility than protons or photons, though their immunomodulatory and regenerative effects remain an area of active investigation [130–132].

The effects of ionizing radiation on the bone microenvironment are highly dose dependent. Low-dose radiation (typically < 2 Gy) can modulate the TME without overt cytotoxicity, inducing transient endothelial activation, promoting antigen presentation, and enhancing immune infiltration-effects that may be harnessed to sensitize tumors to immunotherapy [133, 134]. In contrast, high-dose radiation (> 8–10 Gy), while effective in tumor debulking, triggers widespread stromal remodeling, including osteoblast and osteoclast dysfunction, marrow adipogenesis, neural damage, and hematopoietic suppression [135, 136]. High-dose exposure may also exacerbate chronic inflammation and fibrosis, contributing to long-term TME dysregulation and impaired regenerative capacity [136]. Understanding this dose–response continuum is essential for designing rational combination therapies and fractionation strategies that minimize collateral damage while maximizing anti-tumor efficacy.

## Radiation effects on the bone microenvironment

While the increased proliferative rate of cancer cells makes them more radiosensitive than normal cells, radiation can also damage surrounding tissues that are slower cycling or terminally differentiated. Radiation injury of normal skeletal tissue injury manifests as acute dose-limiting toxicity, including local marrow ablation. These acute toxicities can be managed during treatment by dose modification and symptomatic support, while late toxicities, such as bone embrittlement and marrow fibrosis, arise well after the opportunity to adjust the dosage has passed. The incidence of late toxicity syndromes of bone, like other tissues, is a probabilistic function of dose, but with a stochastic latency that can evolve over a period of weeks-to-years following radiation exposure [137]. Therefore, strategies to selectively enhance tumor cell death (radiosensitization), or selectively prevent toxicity to normal tissues (radioprotectants) or support restoration of normal tissue function, are needed. However, a more nuanced approach, which exploits the transient effects of radiation through combination treatment strategies while minimizing the long-term side effects of radiation may be warranted. Here we highlight the effects of radiation on non-tumor cells in the bone microenvironment, calling attention to the temporal aspects of these changes with the hopes that this knowledge can inform therapeutic approaches.

### Vascular remodeling

The bone vasculature can be subjected to immediate radiation injury and delayed changes affecting tissue physiology and function (Fig. 1). A well-established sequence of vascular changes following radiation has been described for many tumors and normal soft tissues, but is less well understood for bone. Numerous studies outside of the bone have demonstrated that radiation transiently destabilizes the endothelial barrier,

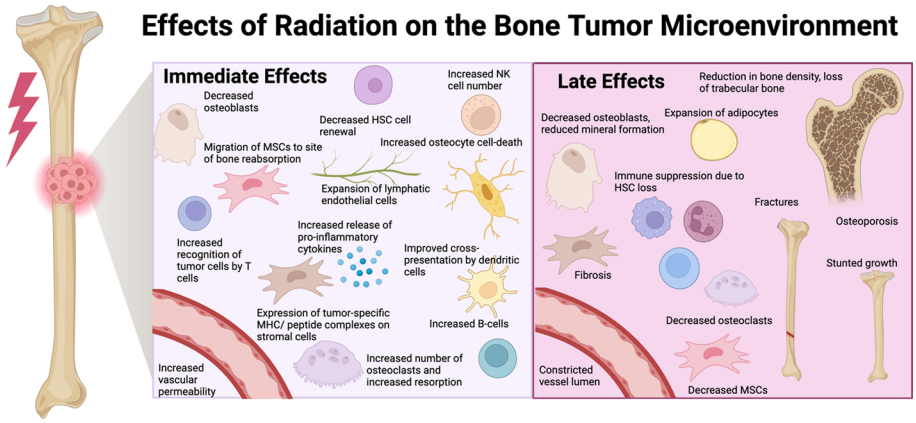



**Fig. 1** Effects of radiation on the bone microenvironment. There are several changes that occur in the bone microenvironment in response to radiation. These changes can be transient or sustained. Immediate changes to the bone microenvironment include loss of osteocytes [166] and osteoblasts [129] and a gain of osteoclasts [168] with increased bone resorption. In addition, post-radiation there is a transient increase in vascular permeability [144] and expansion of lymphatic endothelial cells [38]. Stromal response is also an immediate action. Stromal cells transiently express tumor specific peptides, promoting anti-tumor immunity. In addition, MSCs migrate to sites of bone reabsorption [52]. Concurrently, there is an increase in inflammation and immune activation, mediated by the release of inflammatory cytokines such as TNF-a and IL-1B [147]. Further, there are increases in NK cell and B-cell number [154] in addition to improved tumor cell killing by T cells shortly after radiation treatment [46]. Late effects of radiation treatment include loss of osteoclasts, in addition to sustained loss of osteoblasts [129], which begins immediately after radiation treatment. Osteoblasts display reduced bone-forming capabilities post-radiation. Changes in the number of osteoblasts and osteoclasts [166] may be in part responsible for the long-term side effects of radiation, such as increased propensity for fractures, reduction in trabecular bone, osteoporosis, and stunted bone growth. Other late-stage changes in the bone microenvironment include reduced vessel diameter [138] and immune suppression, which is thought to be secondary to the loss of HSCs [216], which occurs immediately after radiation treatment and increased BM adiposity [166]. This diagram should be taken as a launching point into the investigation of radiation-mediated changes in the bone microenvironment, and not an exhaustive list. HSC, hematopoietic stem cell; MSC, mesenchymal stromal cell; MHC, major histocompatibility complex. Figure created using Biorender; https://BioRender.com/oaan36a

resulting in increased vascular permeability of capillaries [138]. Mechanistically, radiation-enhanced vascular permeability has been attributed to alterations of signaling through inflammatory pathways [139–141], including the PKC, MPK/NF-kB, and sphingosine-1-phosphate pathways [142], leading to cytoskeletal rearrangement and transient disengagement of VE-cadherin junctions [143]. Murine models of radiation injury in the bone have demonstrated transient alterations in the expression of cell adhesion molecules, such as PECAM, and solute carriers, Slc22a14, Slc4a1, Slc7a11, Slc30a10, and Slc16a10 in bone marrow (BM) endothelial cells immediately after 5 Gy of total body radiation [144]. Radiation also induces delayed and long-term effects on the vasculature, such as permanent alterations in vascular paths, vessel wall thickening, constriction of the lumen, and fibrotic remodeling [138]. The ubiquity of these findings suggests that the response to ionizing radiation observed in other tissue contexts would be conserved in the setting of bone vasculature. Mandibular osteoradionecrosis is thought to be a consequence of ischemia due to radiation injury of the inferior alveolar artery as the primary blood supply [145]. The transient radiation induced vascular permeability may be exploited through combination approaches to improve delivery of therapies and overall anti-tumor efficacy. In addition, heightened vascular permeability can enable increased immune cell infiltration to the site

of the tumor [146], suggesting that combining radiation with immunotherapies may improve their efficacy.

A study by Biswas et al. [13] showed that lymphatic vessels, located in the cortical bone, respond to radiation injury by proliferating and expanding. By 14 d after radiation exposure, the lymphatic vessels return to pre-radiation levels. Using light sheet microscopy, selective lineage depletion, and lineage tracing, the study reported that expanding lymphatic vessels secrete CXCL12, promoting the expansion of pericytes that differentiate into osteoblasts and support hematopoiesis. This investigation highlights the notion that radiation induces changes in the bone microenvironment that synergistically impact several key cellular players.

#### Activation of immune response

In response to radiation, the immune milieu of the bone microenvironment itself is altered (Fig. 1). Radiation induces the release of pro-inflammatory cytokines including TNF-a, IL1B, and TGFb [147]. A study by Wang et al., reported that 12 Gy of radiation resulted in increased activation of the STING-pathway and the expression of NF-κB and IL-12 in the bone microenvironment. In addition, radiation induces the release of damage-associated molecular patterns (DAMPs) that promote the activation of an innate immune response [148]. It was also shown that dendritic cells and macrophages experience low rates of apoptosis at low doses of irradiation (0.5 and 1 Gy), while the precursory monocytes experience higher rates of apoptosis within this dose range due to increased oxidative stress [149]. Given that tumor associated monocytes populations are often immune suppressive [150], the depletion of these populations in the setting of bone tumors may be therapeutically beneficial. However, given that monocytes are the precursors of osteoclasts [151], the loss of this population may contribute to long-term bone remodeling dysfunction after radiation [152].

The adaptive immune system is also activated in response to radiation. For example, a study by Zebertavage et al. demonstrated that radiation induces increased recognition of tumor cells by T cells [116]. The pro-inflammatory and immunogenic cell death that is generated via radiation improved dendritic cell cross presentation, increased the diversity of T cells targeting the target, and promoted T-cell trafficking [153]. The relative number of B cells and NK cells also increase [154] in response to radiation, supporting the mounting of an adaptive immune response. These phenomena are thought to contribute to the abscopal effect, or the shrinking of tumors outside of the local plane of therapeutic delivery, which is of particular benefit to patients with metastatic disease. However, radiation also locally depletes HSCs in the BM and can lead to long-term hematopoietic failure, including reduced capacity to develop several immune cell subsets, which may result in impaired immunity [155]. Taken together, radiation treatment is immunomodulatory, and there is a window of opportunity to take advantage of this activity through combination strategies. However, several studies have demonstrated that this immune activation is dose-dependent and lesion-dependent [156]. Determining the optimal conditions to stimulate increased anti-tumor immunity, and subsequently the abscopal effect, is currently an area of active investigation [156, 157].

### Tipping the balance between mesenchymal stromal cells and adipocytes

Moreover, mesenchymal stromal cells (MSCs) and adipocytes are also altered by radiation, and these changes can impact bone remodeling and hematopoiesis. Using human BM-MSCs, it was found that 0.1 Gy γ-irradiation ($^{137}$Cs-based) delayed the expansion of the BM-MSCs, but there were no changes observed in adipogenic or osteogenic differentiation potential [158]. In another study, irradiation of human BM-MSCs with 2.5 Gy Cs-based γ-irradiation increased osteogenic differentiation and MSC support of hematopoietic cells but decreased adipogenic differentiation [159]. A study by Kim et al. demonstrated that MSCs helped to mitigate the radiation damage incurred by the hematopoietic progenitor/stem cells, evidenced by a lack of DNA damage and lower apoptosis rates in protected hematopoietic progenitor/stem cells [160]. This study found that Notch2 signaling played an essential role in mediating the radioprotective effect of MSCs on hematopoietic progenitor/stem cells [160]. Several studies using BM-MSCs derived from either human or rat sources have found that higher doses of radiation ($\gtrsim$ 5 Gy) increased the rates of apoptosis in BM-MSCs and decreased their adipogenic and osteogenic potentials [161–164]. In primary rat BM-MSCs, this impairment was brought on through miR-22-mediated oxidative stress through a decrease in SOD2 activity and an increase in Nox4 activity [162–164]. This response is independent of early growth response 1 (EGR1 ), a tumor suppressor that regulates HSC quiescence, proliferation, and mobilization [161]. There is evidence that the decreased proliferative and differentiation potential of MSCs brought on by radiation is contributed at least in part by senescence and G2 mitotic arrest [163], but it is possible that cells that are mitotically arrested during the time of irradiation are still capable of differentiation [162].

Another common long-term effect of radiation that is poorly understood is the increase in bone marrow adiposity. Because bone marrow houses MSCs that give rise to osteoblasts, increased bone marrow adiposity may translate to decreased osteogenic capacity. Post-radiotherapy marrow adiposity has also been shown to inhibit engraftment of HSCs as well [165], which may contribute to diminished osteoclast numbers following irradiation. Increased marrow adiposity decreases the overall cellularity of the marrow, with adipocytes occupying the limited space that is normally reserved for MSCs and other cells of the bone marrow. Unfortunately, the mechanisms by which this occurs are unclear.

### Dysregulated bone remodeling

Radiation also directly disrupts cellular bone remodeling by damaging osteoblasts, osteoclasts, and osteocytes (Fig. 1). To study the effects of radiation on osteoblasts, Cao et al. irradiated the left distal femur of male C57BL/6J mice using a small animal radiation research platform (SARRP) system, and the effects of radiation were evaluated at distal and proximal locations in both the irradiated and non-irradiated contralateral femur [129]. Histomorphometric analyses of these locations showed a decrease in osteoblast numbers at the irradiated distal left femur, but not in the proximal or contralateral femora [129]. Using a Sprague–Dawley rat model with the SARRP system, Chandra et al. similarly demonstrated that the incidence of apoptotic osteoblasts increased from approximately 5% in non-irradiated bone to approximately 34% in irradiated bone [166].

These studies demonstrated a local loss of viable osteoblasts following focal irradiation of trabecular bone, which may contribute to loss of mineralization potential and subsequent increased bone fragility. In contrast, Oest et al. reported no loss of mineral apposition and cortical thickening for up to 26-weeks following fractionated (4×5 Gy) limited-field exposure mouse hind limb of female Balb/C mice [152].

Osteoclast numbers and resorptive activity also increase transiently post-radiation, though the response may be biphasic. Cao et al. [129] and Chandra et al. [166] both reported a decrease in osteoclast number 1 month following radiation. Mice exposed to 2 Gy X-ray or γ-irradiation showed elevated osteoclast markers (Nfatc1, Csf1, Tnf, Rankl), returning to baseline within 3 d [152, 167–169]. Oest et al. found similar changes in osteoclast number, and have further demonstrated a persistent depletion of osteoclasts, resulting in the accumulation of microdamage due to diminished remodeling capabilities [152]. While the general theme of osteoclast loss following radiation exposure was in agreement between these studies, it is unclear how the differences in animal models and radiation delivery between these studies may contribute to the observed differences in osteoblast activity, and may warrant further research.

Though osteocytes are traditionally considered radioresistant owing to their terminal differentiation, evidence suggests variable responses depending on dose, species, and methodology. Rabbits receiving 15–40 Gy $^{60}$Co irradiation showed minimal osteocyte loss [170], while rats exposed to two 8 Gy fractions had a 4.4-fold increase in empty lacunae—an effect mitigated by PTH (1–34) treatment [166]. High-dose electron beam radiation also reduced RNA synthesis in osteocytes for up to 52 weeks, indicating persistent metabolic dysfunction [171]. Collectively, these studies suggest osteocyte injury contributes to chronic remodeling defects post-radiation.

## Radiation and combination therapy in bone tumors

Radiation is often used clinically with other treatments such as chemotherapy or bone tumor surgery. However, employing additional strategies in combination with radiotherapy may improve outcomes for patients. Two primary goals of combination strategies in radiobiology are radiosensitization and radioprotection. However, radiation can also prime the TME for additional therapeutic modulation through immunotherapies (Fig. 2).

### Radiosensitization

Radiosensitization refers to therapies which selectively enhance tumor cell death. In the setting of bone tumors, there have been several therapeutic approaches for radiosensitization investigated (Fig. 2) [172]. For example, mithramycin A has shown great promise as a radiosensitizer for Ewing's sarcoma, by hindering double strand break repair. Kim et al. demonstrated that zoledronic acid selectively radiosensitizes osteosarcoma cells by inducing reactive oxygen species (ROS) production and a reduction in DNA repair enzymes such as ATR, DNA-PK, and Rad52 [173]. This treatment has shown great promise in the clinic, but there is still need for patients who do not respond to this therapy. In addition to increasing tumor cells' sensitivity to radiation, there is also interest in radiobiology to protect bone microenvironmental cells.

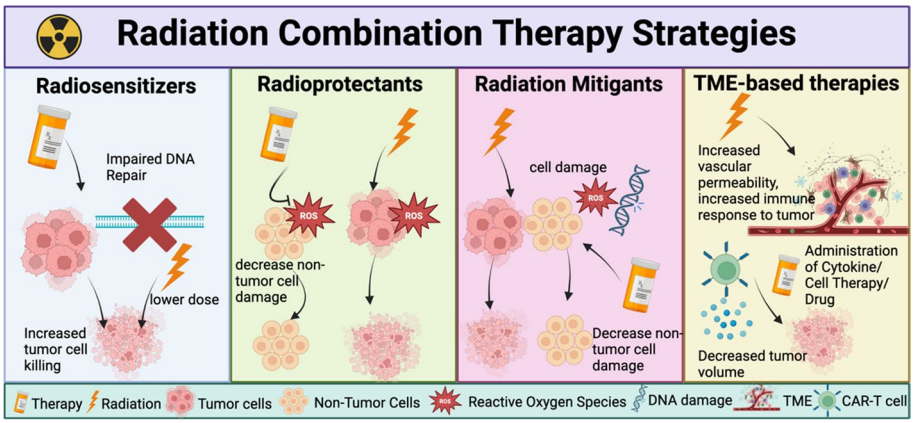



**Fig. 2** Radiation as a combination therapy. Radiation can be used in combination with several therapies to enhance anti-tumor efficacy or to limit off-target toxicity. Radiosensitizers are administered prior to or with radiation treatment to make tumor cells more sensitive to radiation [172]. For example, some radiosensitizers impair DNA repair in tumor cells. Radioprotectants are also given prior to or at the same time as radiation therapy [68]. However, these therapies decrease off-target effects in non-tumor cells, such as by reducing ROS production in non-tumor cells. Radiation mitigants are given after radiation to prevent damage to non-tumor cells [68]. These therapies repair damage to non-tumor cells. Radiation can induce changes to the TME. TME-based therapies may be delivered after this modulation occurs to elicit a stronger anti-tumor response [153]. Figure created using Biorender; https://BioRender.com/oaan36a. TME, tumor microenvironment; ROS, reactive oxygen species; CAR-T cell, chimeric antigen receptor T cell

### Radioprotection and mitigation

Radioprotection refers to therapeutic strategies that selectively prevent toxicity to normal tissues or support restoration of normal tissue function (Fig. 2). Radioprotectant agents are delivered either before or during radiotherapy [174, 175]. It is important for the radioprotectant to be selective for normal tissue. If the radioprotectant also prevents radiation damage to tumor cells, its usefulness in a clinical setting is reduced. The majority of radioprotectants have some type of antioxidant capability, and function by neutralizing any free radicals or reactive oxygen species that may otherwise damage the cell [174, 175]. Amifostine is currently the only clinically used radioprotectant and is employed primarily in conjunction with head and neck irradiation to reduce the mucosal side effects of radiation, particularly xerostomia (dry mouth) [176]. The selectivity of amifostine is pH dependent. Whereas tumor microenvironments are generally below pH 7.2, the neutral environment of normal tissues favors metabolic conversion of the prodrug (WR-2721) by alkaline phosphatase, favoring accumulation of the free thiol (WR-1065) that functions as a free radical scavenger, thereby reducing the amount of DNA damage that occurs in normal cells [177, 178] and improving efficiency of DNA repair by HR [179]. Early studies in rat models showed that amifostine reduced the adverse effects of radiotherapy on bone growth rate, growth plate height, matrix accumulation, and limb length, through initial preservation of PTHrP expression and decreased Bax expression [180].

Radiation mitigants are compounds that are implemented either during radiotherapy or shortly thereafter and seek to reduce normal tissue injury before any clinical presentation of damage occurs (Fig. 2) [174, 175]. These treatments involve the use of cytokines, growth factors, and hormones to promote DNA damage repair in cells and increase cellular proliferation [174, 175]. Bisphosphonates are the most widely prescribed drug

class used to combat osteoporosis. Bisphosphonates have a high binding affinity for the hydroxyapatite crystals found in bone [181]. Bisphosphonates lead to osteoclast apoptosis [182] and decrease bone resorption. Patients demonstrated increased bone density following treatment with radiation and either disodium pamidronate or ibandronate [183, 184]. These studies demonstrate the efficacy of bisphosphonates in improving bone density and reducing the incident rates of fragility fractures following radiation therapy.

#### Radiation and TME-based therapies

Here we outline how radiation can be used as a TME modulator and how it can be combined with other TME-modulators to elicit systemic anti-tumor responses (Fig. 2). The activation of anti-tumor immunity mediated by radiation may be further capitalized to achieve the abscopal effect in solid tumors through combination with immunotherapies [156, 185]. Combination radiation and immunotherapy is increasingly being explored in the clinic for the treatment of bone tumors, offering a promising dual approach to combat these challenging diseases. However, much of what is known about the combination of radiation and immunotherapy stems from other solid tumors. A clinical trial which administered granulocyte–macrophage colony stimulating factor (GM-CSF) in combination with radiation for patients with metastatic solid tumors demonstrated that patients who received this combination approach had increased abscopal responses [153]. Mechanistically, this response is attributed to the immunogenic cell death elicited by radiation. GM-CSF administration is thought to result in the differentiation of dendritic cells which cross-present tumor antigens to T cells, generating tumor-specific effector T cells [153, 185]. Interestingly, the timing, dose, and lesion site all influence the efficacy of these combination strategies. In the setting of primary bone tumors, radiation combined with immunotherapy has been underexplored. In this context, radiation is utilized as a pretreatment for lymphodepletion of T cells to generate a higher number of T cells that can be collected for the generation of CAR-T cells [185]. In addition, immune checkpoint inhibitiors (ICI) combined with radiotherapy has shown benefit in patients with bone metastasis. Further investigating combination approaches of radiation and immunotherapy will undoubtedly yield import clinical insights for patients with bone tumors. In addition to immune modulation, radiation also influences the stromal compartment of the tumor. For example, radiation increases vascular permeability. Given that the vasculature is the gateway to drug delivery in bone tumors, radiation could enable increased drug trafficking to the tumor site by increasing vascular permeability. Exploring the effects of stromal remodeling induced by radiation and opportunities to exploit these changes is also a promising area of investigation.

## MPS models of radiation in the bone tumor microenvironment

The major tasks in the field of bone radiobiology include further exploration of the effects of radiation on the bone microenvironment, minimizing or repairing the long-term side effects of radiation, and using rationale combination approaches to minimize the doses of radiation required to treat bone tumors. The complexity of the bone TME and the dynamic effects of radiation on this ecosystem make these tasks challenging. In this sense, MPS platforms represent valuable tools to further explore the effects of radiation of the bone TME, and strategies to minimize toxicities and develop synergistic

combination therapies [186]. To date, however, use of these systems in the context of bone tumor radiotherapy and radiation-induced bone disease is relatively underexplored. Here we outline the diversity of MPS approaches that could be leveraged to create more physiologically relevant models of bone tumors, with an eye toward application of MPS models to test radiobiological hypotheses in this unique tissue microenvironment.

### Recapitulating the 3D tumor microenvironment of bone tumors

To recapitulate the 3D nature of TMEs, tumor spheroids or 3D cultures of tumor cells are utilized (Fig. 3). Tumor spheroids better recapitulate the physiological microenvironment of a tumor because they can mimic gradients of oxygen and pH that are observed in vivo [187, 188]. In addition, spheroids can mimic the necrotic core of in vivo tumors in addition to zones of proliferation and quiescence [188]. Furthermore, tumor spheroids are often derived from a single clone and display spatially distinct patterns of gene expression, like those observed in patient tumors obtained on biopsy [187]. In addition, co-culture systems in the setting of radiation can further model the effects of radiation-induced cell death, radioresistance, and more. A study by Brunigk et al. demonstrated that the response to radiation (0–30 Gy) combined with hyperthermia more closely mimicked that of in vivo responses compared with monolayer cultures of colon cancer

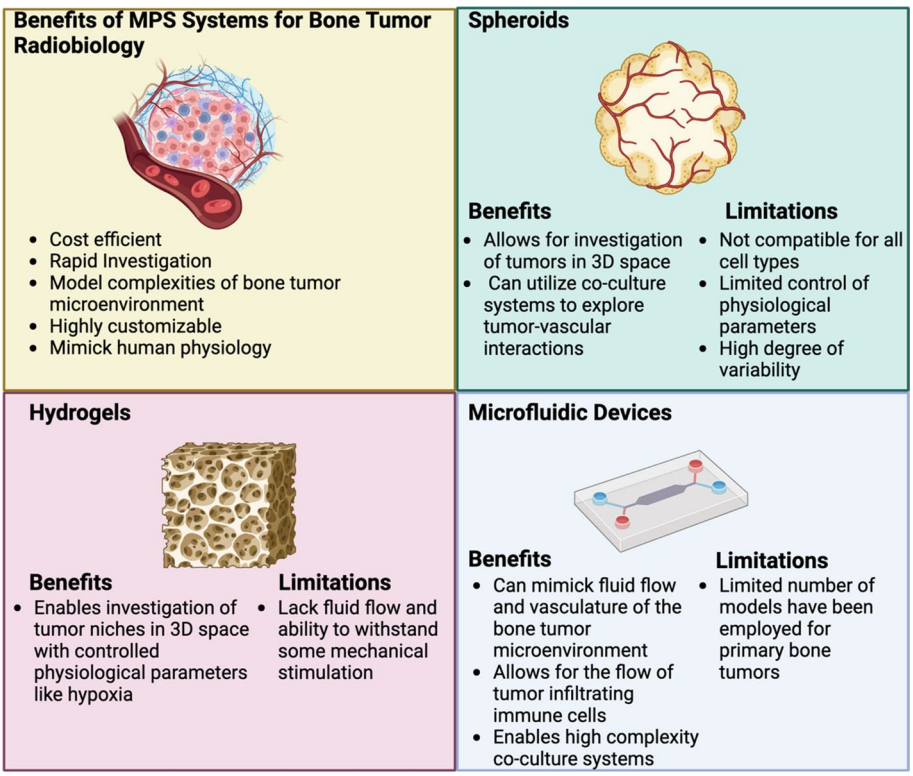



**Fig. 3** MPS to probe radiobiology in bone cancer. MPS, including spheroids [187, 188], hydrogels [190], and microfluidic devices [202], allow for rapid investigation into the effects of radiation on bone tumors. These systems are uniquely equipped to model the complex microenvironment of bone tumors in a rapid, cost-efficient, and scalable manner [186]. Each modality within MPS has its own benefits and limitations. Figure created using Biorender; https://BioRender.com/oaan36a

and squamous cell carcinoma cell lines [189]. For example, tumor spheroids demonstrated a higher propensity for regrowth after treatment compared with monolayer culture. One challenge of tumor spheroids is that not all tumor cell lines, or patient derived cells, can readily form 3D spheroids. Variation between the sizes of the spheroid can also induce experimental variability.

To overcome the challenge that not all cells can form spontaneous tumor spheroids or organoids in 3D cultures, hydrogels can be used to provide structural support and a regulated microenvironment (Fig. 3). Hydrogels are polymeric, hydrated 3D materials, which can be utilized as a tissue scaffold for three-dimensional culture systems to more accurately mimic the TME. Importantly, studies have demonstrated that hydrogel environments influence radiotherapy resistance [190]. In addition, hydrogels can enable investigation of 3D bone tumor micro-niches [191]. Several investigators in the field of bone sarcomas have utilized hydrogels [192]. A study by Juriga et al. demonstrated that poly(aspartic acid)-based hydrogels are a viable scaffolding option for 3D osteosarcoma culture [193]. Hydrogels can also enable rapid investigation of therapies for bone metastases that can be challenging to model in vivo. A study by Fong et al. demonstrated that hyaluronic acid-based hydrogels can be employed for 3D culture of patient-derived prostate cancer cells to assess response to chemotherapy [194]. Montiero et al. developed a humanized 3D osteosarcoma (OS) model and studied tumor invasion by encapsulating spheroids in methacryloyl platelet lysates (PLMA)-based hydrogels with osteoblasts and mesenchymal stem cells co-cultures [195]. When subjected to doxorubicin treatment, this model showed improved drug resistance as compared with scaffold-free spheroids. Villasante et al. developed a new model of Ewing's sarcoma (ES) by culturing osteoclasts and osteoblasts within a three-dimensional mineralized bone matrix, showing that that ES cell aggregates induced decreases in bone density, connectivity, and matrix deposition, while the use of zoledronic acid inhibited osteoclast-mediated bone resorption [196]. Molina et al. studied invasion and Ewing's sarcoma (ES) pathogenesis using a 3D bone tumor niche generated from decellularized electrospun poly(ε-caprolactone) PCL scaffolds and osteogenic human mesenchymal stem cells [191]. This study showed that compared with 2D cultures, 3D environments facilitated the downregulation of key targets of recent clinical trials, namely, canonical insulin-like growth factor 1 receptor (IGF-1R) and rapamycin (mTOR). Such models could be used to generate cell phenotypes that were resistant to mTOR inhibition and chemotherapy. A similar approach using hydrogel scaffolds can be used in radiotherapy settings, especially for investigating radiosensitizers [197]. In addition, hydrogels can also support co-culture systems to investigate the effects of radiation or other therapies on TME cells. These systems can include tumor and normal stromal-vascular and parenchymal cells to replicate 3D interactions between cellular populations [198] and how this influences therapy response and resistance. These systems can also model paraneoplastic changes caused by tumor cells in the bone microenvironment [199].

#### Microfluidic devices

While tumor spheroids and organoid cultures with or without hydrogel matrices enable 3D investigations that more accurately represent the physiology of tumors, reproducible application of mechanical stimuli remains a challenge [191]. Furthermore, they lack the

ability to mimic fluid flow, mechanical loading, and other physiologically relevant processes affecting the bone microenvironment, factors that are critical to holistically assess the effects of radiation therapy and combination strategies in the context of the bone tumor microenvironment.

Microfluidic devices can fill in the gaps of hydrogel and spheroid/organoid culturing methods (Fig. 3) [36, 200, 201]. Microfluidic devices are small instruments containing tumor cells (which may be co-cultured with stromal-vascular and parenchymal cells and hydrogel matrices) that enable the constant perfusion of media, nutrients, and experimental drugs [202]. These devices can also be multiplexed to incorporate human-derived organoids representing several tissues on the same device, potentially accelerating absorption, distribution, metabolism, excretion, and toxicity (ADMET) testing. Multiple microfluidic devices can be linked together to construct multi-organ devices, which can enable investigation of immune cell recruitment, metastasis, and other physiological processes [203, 204].

The application of microfluidic systems to model the bone tumor microenvironment is beginning to unlock fundamental insights of events occurring at the tumor–vascular interface. In the setting of bone tumors, these devices have been employed to study key metastatic processes including tumor cell extravasation [200], organ-specific metastasis [205], tumor cell circulation and homing [164, 206], and metastatic priming of the bone microenvironment [207], as well as investigation of cellular immunotherapies. Jeon et al. developed a microfluidic chip to analyze the process of human breast cancer cell extravasation into bone-mimicking microenvironments through a microvascular network [200]. Such compartmentalized models can be an effective drug screening platform, as well as help identify new molecular pathways involved in cancer biology. Fevre et al. developed a platform based on anchored droplets to generate and monitor growth of spheroids of A673 Ewing sarcoma (ES) cells followed by etoposide and cisplatin treatments in a user-defined manner. Such droplet-based microfluidics offer modularity and high-throughput drug screening [83]. Bonnett al. developed a cancer-on-a-chip platform model to evaluate the impact of adenomatous polyposis coli (APC) mutations on cytotoxic T lymphocyte (CTL) migration and cytotoxicity against 3D tumor spheroids [208]. Results show that APC mutated CTLs are found to have a reduced ability to destroy tumor spheroids compared with control cells. Such chips could help identify the steps that limit tumor destruction. Jaiswal et al. used extrusion-based 3D bioprinting to develop a new osteosarcoma model by incorporating both tumor and stromal components in the presence of physiologically relevant mechanical stimulation [82]. Drug screening with this dynamic model showed enhanced sensitivity to anticancer drugs (doxorubicin, cisplatin, sorafenib) as compared with static conditions, highlighting the need to develop physiologically relevant complex models for accurate preclinical screening of anticancer drugs.

Microfluidic models are also revealing insights into how the mechanobiologic aspect of the microenvironment may impact the biology of metastasis [209] and response to treatment [210, 211]. A study by Tratchenberg et al. using microfluidic devices of Ewing's sarcoma demonstrated that sheer stress gradients can influence tumor cell proliferation and recapitulate tumor heterogeneity [212]. While microfluidic devices have primarily investigated metastasis, these approaches can be further expanded into the fields of primary bone tumors and radiobiology [186]. For example, this technology can be utilized

to further delve into the effects of radiation on bone vasculature and to model changes that occur in recruited immune populations. Furthermore, these systems can be used to assess the therapeutic efficacy of combination strategies which target multiple TME cellular populations in the setting of a controlled and physiologically relevant niche.

#### Application of engineered microenvironments to study the radiobiology of bone tumors

The physiologic state of the tumor microenvironment, particularly in terms of tumor vascularization and oxygenation, are key determinants of radiosensitivity that are difficult to replicate in vitro. Therefore, the field of radiobiology has historically relied upon animal models that are costly, low throughput, suffer from poor reproducibility, and have demonstrated limited translatability owing to unforeseen toxicity that emerges in human trials. The net result of this reliance on animal models is that significant investment of time and resources are lost, demonstrating a need for more physiologically relevant model systems to screen and validate candidate radiation-modifying agents.

Engineered tissue microenvironments, as discussed above, may be a part of the solution to these problems, but few studies have utilized tissue-engineering approaches to test radiobiological hypotheses, particularly in the context of the bone-tumor microenvironment [197]. Here we summarize the current state of tissue engineering approaches to study the radiobiology of the bone-tumor microenvironment. Bavoux et al. combined microfluidic platforms with radiotherapy to screen toxicity of 16 drug–radiotherapies in cancer soft tissue sarcoma (STS) spheroids from 336 patients [213]. Such models could be used to screen libraries of pharmaceutical compounds to identify optimal molecular agents and radiotherapy dosages. To provide insights into the effects of radiation exposure occurring during deep-space missions, Tavakol et al. developed a duplex (bone and cardiac) microphysiologic system [214]. Following exposure to neutron irradiation, they evaluated the phenotype and function of marrow cells, as well as changes on the expression of inflammatory, oxidative stress, and matrix remodeling genes in heart and bone, discovering that bone marrow-derived inflammatory cells contribute to hypertrophic remodeling of the heart during extended space travel. Such models could allow for testing of radioprotective measures. Chermat et al. developed a brachytherapy (BT)-on-a-chip model involving the manual insertion of BT seeds to replicate TG-43 formalism, allowing the modulation of clinically relevant conditions such as dose rate and tissue oxygenation, which affect radiosensitivity [215]. Choi et al. used a microvessel-on-a-chip to study radiation-induced vascular dysfunction, finding that radiation led to a reduction in vascular structures, suppression of blood vessel recovery, and loss of angiogenic ability. In the future, it is anticipated that MPS will be employed in high-throughput screening of candidate radiation modifying agents and multiplexed to simultaneously evaluate the responses of tumors and normal tissues, accelerating the pace of translation of novel agents to the radiotherapy clinic.

## Summary and looking forward

In summary, the bone is a complex microenvironment that can house primary and metastatic tumors with significant clinical impact. One of the primary means of treatment for bone tumors is radiation therapy. While radiation most readily damages tumor cells, the bone microenvironment can also sustain injury from radiation. There

are several transient effects of radiation, most notably vascular permeability and increased immune activation. Investigating this therapeutic window of opportunity can yield improved outcomes for patients with primary and metastatic bone tumors. Moreover, the long-term impacts of radiation can be detrimental to the quality of life of patients. Further elucidating the cellular mechanisms underneath these off-target effects may yield insights into preventative measures.

There is an opportunity to exploit the impacts of radiation therapy through combination treatment strategies that result in more durable therapeutic responses and that minimize treatment limiting toxicities. Current combination strategies focus on increasing radiosensitivity and protecting or mitigating non-tumor cells from radiation induced damage. While there has been immense success with several of these strategies, there is still room for improvement in these therapies. Looking forward, with the increase in immunotherapy and investigations into the TME, there will be increasing utility for understanding how radiation can prime the TME for additional therapies. Areas of future direction include choosing the appropriate dose, lesion site, and timing for radiation and immunotherapy combination approaches. In addition, exploring the therapeutic utility of the radiation-induced modulation of the stromal compartment in bone tumors is also an area ripe for future exploration.

MPS models serve as an ideal approach for efficient investigation of radiation and combination strategies in the bone microenvironment. Modeling radiation response in bone tumors using MPS involves several critical considerations. The complexity of bone tissues, with their heterogeneous structure and composition, must be accurately represented. Incorporating various cell types found in the bone, including stromal and immune cells, is essential for capturing the dynamic interactions that influence radiation response. Furthermore, the physiological microenvironment, including factors such as hypoxia and nutrient gradients, should be simulated to understand their impact on radiation sensitivity. MPS systems should also account for the mechanical properties of the bone, such as stiffness and elasticity, which affect cellular behavior and radiation response. In addition, temporal aspects, such as the timing of radiation exposure and subsequent cellular responses over time, must be modeled to evaluate long-term effects accurately. Addressing these considerations ensures that MPS systems provide a robust platform for studying radiation responses in the bone microenvironment, offering insights that can guide therapeutic strategies and improve clinical outcomes.

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**Author contributions**
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**Data availability**
No datasets were generated or analyzed during the current study.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
Pranav Soman founded and is sole owner of 3D Microfluidics, LLC.

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