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PRECLINICAL ANIMAL MODELS IN REGENERATIVE MEDICINE (D HUTMACHER, SECTION EDITOR)

Human Bone Xenografts: from Preclinical Testing for Regenerative Medicine to Modeling of Diseases

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Abstract Xenografting involves the transplantation of human tissue or cells into animal models and is an important tool for regenerative medicine research. Implantation of engineered human bone tissues into animal models, for example, is performed in preclinical evaluations of product safety and efficacy. With the advent of improved experimental methodologies, these models are further being exploited to interrogate molecular mechanisms and physiological interactions in vivo. In parallel to these developments, patient-derived xenograft murine models of cancer are increasingly being studied for various applications in cancer research and therapy; it follows that xenograft models in tissue engineering may be adapted for such approaches. In this review, we first discuss the development of human bone xenograft models to recapitulate physiological states in regenerative medicine. Subsequently, we discuss the use of these techniques for applications in modeling pathological states in skeletal oncology, namely, hematopoietic malignancies, bone metastatic disease, and primary bone malignancy.

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Introduction

Xenografting into animal models is commonly performed in the preclinical evaluation of engineered tissues for regenerative medicine, with the major objective of demonstrating safety and efficacy of the engineered constructs for regenerative and reconstructive applications. These range from small animal models, where the engineered tissue constructs are typically implanted in an ectopic location, to large animals, where they are usually implanted orthotopically [1], with many experimental tools specifically designed for the evaluation of physiological responses to the implanted constructs in vivo. For example, novel and powerful imaging modalities have been developed to observe vascular development, in order to shed light on angiogenic process following implantation of engineered bone tissues [2, 3]. Advances in multimodal imaging have also made it possible to concurrently monitor such responses in parallel [4] and/or multiple cell types homing and migrating to the implanted constructs [5].

Beyond applications in regenerative medicine, human bone xenografted models have also proven useful as experimental models of skeletal disease, by providing a platform to study human-specific pathologies. Leveraging on the customized experimental techniques described above, these models provide unique opportunities to study disease states in vivo, which would otherwise be impossible on human subjects. Such use of human-derived tissue is of particular value in characterizing species-specific behavior and provides specific insights into disease etiology and drug efficacy over existing animal disease models. This review thus focuses on the use of xenografted bone tissues as experimental models in musculoskeletal oncology, beginning

with a brief overview of xenografted bone models for regenerative medicine, and leading to current studies in the use of engineered bone tissue for the study of bone marrow-residing leukemias, bone-metastatic cancers, and bone-primary tumors.

Engineered Human Bones in Xenograft Models: Preclinical Testing for Regenerative Applications

Animal Models for Bone Regenerative Studies

A wide range of mammals have been used for preclinical evaluations of tissue-engineered bones. Small rodents such as mice and rats are generally preferred in early-stage studies where large numbers are required [6]. Additionally, genetically engineered mice (GEM) are available that provide a plethora of experimental settings for study design, including diseased phenotypes and, more specifically, immunocompromised mice for xenografting studies [7, 8]. However, rodent models are limited in the space and volume of implants afforded, and implantation is commonly performed ectopically in the subcutaneous pockets or peritoneal cavity.

In the evaluation of regenerative capacity, orthotopic implantations are preferred and most commonly performed on larger animals such as rabbits, sheep, and dogs [9, 10]. In these models, a critically sized, non-healing defect is surgically introduced into the animal skeleton and subsequently treated with the engineered tissue graft. Such models are isomorphic to the clinical situation and purportedly demonstrate better approximation to human bone biology and composition, as compared to their small animal counterparts [11].

Defect type and location are also varied to reflect different clinical scenarios or to facilitate specific experimentation. For example, segmental defects in load-bearing long bones, such as the femoral and tibial diaphysis, more accurately reflect the mechanical loads experienced in vivo. [12–14]. Such defect sites have well-defined geometry and location, facilitating studies for the tracking of new bone formation and vascular infiltration and mechanical testing for the evaluation of torsional torque and bending strength as measures of functional recovery [15]. In contrast, non-load-bearing facial/cranial defects may be employed that usually do not require additional fixation. These models are less challenging technically and more amenable to small animal experimentation. Additionally, the absence of (usually metallic) fixtures avoid confounding effects of biomechanical stress shielding [16] and render the surgical site more amenable to radiographic imaging, facilitating serial, non-invasive monitoring.

Evaluation Reparative Outcomes

The accurate evaluation of physiological responses represents another major challenge in the design and conduct of experiments with xenografted animal models. Mineralization and/or bone bridging in the bone graft, as determined by the mineral extracellular matrix (ECM) formed, is most often taken as a primary indicator of bone healing and is most readily observed through radiographic imaging. This is commonly performed with portable imaging systems (such as C-arms) to provide gross observations, while computed tomography (CT) techniques have gained popularity for high-resolution three-dimensional reconstructions, which allow the acquisition of quantifiable parameters in the region, including volume and area of mineral tissue [17]. Histological staining of sample sections is also commonly performed at end-point. Hematoxylin and eosin (H&E) stain is mostly used, which allows to observe not only ECM formation, but also the ingrowth of cells. Von Kossa staining and Masson's trichrome staining are also commonly used to identify mineral phosphates and ECM organization [18]. Immunohistochemistry techniques may be further performed to allow the specific detection of genes or cell types, providing evidence for mechanistic explanations and findings.

In addition to morphological observations, biomechanical tests are often performed to quantify the restoration of structural function to the fracture site. Depending on implant site, these include torsional, bending, and push-out tests, which are performed on the extracted tissue samples [19]. In order to meet specific requirements for biocompatibility testing, additional protocols for the evaluation of neo-vascularization, chimerism, inflammation, tumorigenesis, and other aspects of safety and efficacy are also readily available [9, 20–23].

Behavior of Engineered Bone Grafts In Vivo

Arguably, the most crucial aspect of bone tissue engineering lies in predicting the performance of engineered grafts in vivo. Ectopic models, most commonly conducted in mouse models, provide a measure of capacity bone formation after implantation. This process is highly dependent on factors intrinsic (osteoinductive and osteoconductive properties) and extrinsic (vascularization, immune reaction) to the graft itself [24]. Provided with adequate conditions, subcutaneously implanted engineered bone grafts have been shown to be capable of engraftment and formation of woven bone [25]. A major advantage of using murine models is the availability of genetically modified mice, in particular immunocompromised strains that demonstrate greater "take" of implanted tissues. Thus implanted, engineered bone constructs carrying mesenchymal and endothelial progenitors are capable of generating osteogenic and vasculogenic tissue of human origin [26•]. Due to technical challenges arising from size limitations, however, these models are not commonly employed in orthotopic settings, which would provide greater predictive value of the efficacy of bone grafts for the treatment of fractures. Thus, ectopic models are typically used instead as a screen in basic

biocompatibility studies; further evaluations in more homologous settings, such as in non-healing, critically sized fracture models, are generally required.

In a review of published articles from January 2000 to November 2015, 175 articles were found in which tissue engineering approaches were used for the treatment of critical-sized bone defects. Among animal species used, rodents were employed in more than 70 % of studies in the field (Fig. 1 b), of which rats were favored for their size and ease of handling, particularly in critical-sized defect models. Anatomical location of the defect site is highly dependent on the choice of animals used (Fig. 1 c); small species such as mice and rats mainly focused on large bones such as cranium and femurs, while the larger animals are amenable for surgery on smaller bones such as radii and metatarsii, in accordance with the study objectives.

The experimental settings of reviewed studies were summarized according to the nature of donor cells (animal origin or human origin) and animal models used (Fig. 1). Across the models, bone marrow-derived mesenchymal stem cells (BMSC) and adipose tissue-derived stem cells (ASC) were used most often, accounting for nearly 70 % of all studies. BMSC were most popularly employed in autologous/

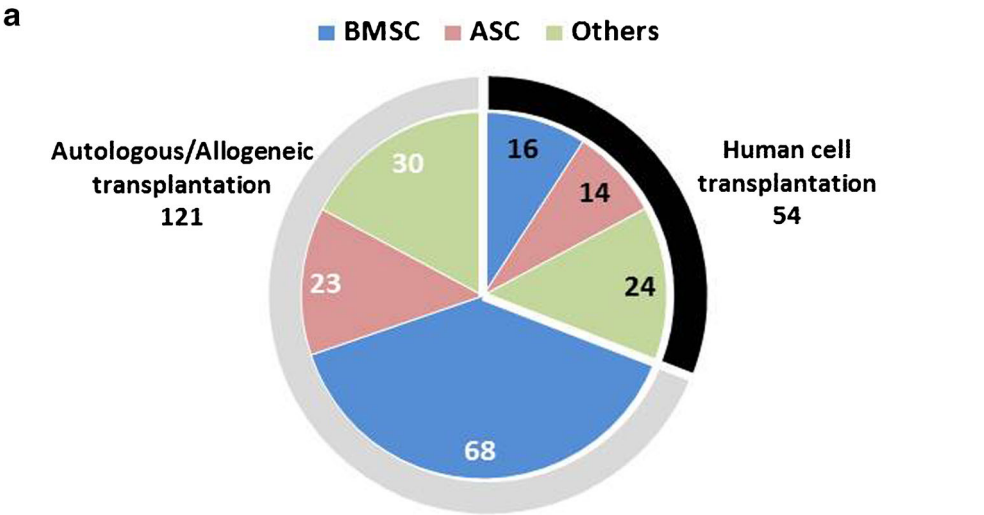

Fig. 1 The experimental settings of reviewed studies were summarized according to the nature of donor cells (animal origin or human origin) and animal models used. **a** The MSC employed in such studies are derived from more accessible tissue, such as adipose, muscle, dental pulp, periosteum, umbilical cord, cord blood, amniotic fluid, and even urine. **b** Among animal species used, rodents were employed in more than 70 % of studies in the field. **c** Anatomical location of the defect site is highly dependent on the choice of animals used

c

Species	Cranium	Mandible	Femur	Tibia	Radius	Ulna	Metatarsus	Iliac wing
Rat	39	2	38	3	-	-	-	-
Rabbit	7	2	3	3	14	1	-	-
Mouse	24	-	3	1	-	-	-	-
Sheep	1	-	2	6	1	-	4	-
Dog	3	5	-	-	1	1	-	-
Pig	2	1	2	2	-	-	-	-
Goat	-	-	1	2	-	-	-	1

allogeneic cell transplantation studies (68 out of 121), but less so in xenogeneic settings. However, this does not imply BMSC to be less suited for xenotransplants, but are simply more easily isolated and better established than their counterparts in allogeneic settings and thus preferred in such experiments [27–29]. In contrast, xenotransplant experiments in regenerative medicine are chiefly performed to evaluate the therapeutic performance of various cell sources for clinical applications (Fig. 1). It follows that the MSC employed in such studies are derived from more accessible tissue, such as adipose, muscle, dental pulp, periosteum, umbilical cord, cord blood, amniotic fluid, and even urine (Fig. 1a). Additionally, xenografting studies using primitive stem cells such as induced pluripotent stem cells (iPSC) and embryonic stem cells (ESC) have also been performed. It should be noted that while these cells provide greater ease of use in the clinical setting [30], they may, in themselves, demonstrate poorer approximation of osteogenic processes in vivo [31].

Findings from the reviewed studies generally suggest significant osteogenesis and healing after implantation of the engineered bone constructs, as compared to the control groups such as an empty scaffold [32], or even against positive controls such as autograft and BMP-2 treatment [33, 34]. About 7 % (13 out of 175) of the studies showed little healing after the treatment with cellular scaffolds, with compromised efficacy ascribed to inappropriate choice of cells or scaffolds, poor conditions, or insufficient amount of cells. Notably, in eight studies using osteoblast cell sources, four showed no improvement to the healing, with two showing positive results only when BMP-2-transduced cell lines were used [35–42].

A major conundrum in preclinical models lies in the conflicting need to evaluate the performance of human-derived cell sources in isomorphic models, against confounding effects of host responses to the xenogeneic cell source. Notably, in studies that compared implantation of human BMSC against autologous cell lines, human cell lines resulted in inferior healing efficacy, even where no inflammation responses was detected [14, 43]. Moreover, many other studies using human cell sources claimed disappearance of implanted human cells after weeks of transplantation, even with strict immunosuppression regimes in place [44–46]. As such, such tests may not be valid as defining models of clinical safety and efficacy, but rather, the experimental results should be interpreted at "risk markers" [47].

Engineered Human Bones in Xenograft Models: Disease Models in Oncology

Bone, as an organ, performs many important biological functions aside from providing structural support, including metabolic homeostasis, hematopoiesis, and stem cell maintenance [48]. Much of these functions are regulated by various niche cells (mesenchymal, endothelial, osteoblast/osteoclast, and immune cells) in the complex, interconnected bone marrow microenvironment [49•]. In recent years, it has become evident that the bone marrow microenvironment nurtures not only normal stem cells (mainly hematopoietic) but also leukemic stem cells [50, 51] and bone metastatic cancers [52], with many of these studies conducted on animals xenografted with human cancer cells.

Transplantation of human cancer (stem) cells or tumor tissue into immunodeficient mice has been performed over the past 50 years. Initially developed as a means of tumor propagation, xenoimplanted tumors are currently being employed in etiological and drug-response studies. Such models offer significant advantages over mice genetically engineered with oncogenes (oncomice) to spontaneously develop cancer, including their ability to capture human cancer-specific behavior [53]. However, in contrast to oncomice, however, the tumors are often ectopically implanted into subcutaneous or renal capsule spaces, which are unable to provide appropriate microenvironmental effects. Even if orthotopically engrafted, given the fundamental differences between human and mice [54•], the murine model might not recapitulate the physiological process of cancer development and stem cell maintenance in human. Bereft of a suitable microenviroment, such models suffer from poor "take" of injected human cancer cells and/or incapability to recapitulate disease features.

Research efforts to generate more "humanized" mouse models have yielded enhanced human cell engraftment, each with their attendant limitations. These include (1) supplementation of selected human cytokines, either by exogenous administration or genetic expression (limited by the absence of stromal cells and difficulties in maintaining cytokine concentrations at physiological level [54•]) and (2) co-transplantation of human mesenchymal niche cells into the mouse bone marrow (limited by an inability to maintain the xenoplanted mesenchymal cells in vivo for a long term [55, 56]). More recently, tissue engineering approaches have been developed to create human bone organoids in mice, in an effort to provide the humanized environment to accommodate human cancer cells. Here, we summarize the recent advances involving the use of engineered human bone in murine models, especially in the context of skeletal oncology.

Hematopoietic Malignancies

Experimental models of leukemia are the most commonly studied xenograft models, due largely to the ready availability of patient cancer cell samples. A major limitation in the use of these models, however, lies in the low xenograft efficiency [57]. Even with positive "take," many engrafted cancer cells display a lack of propensity for expansion, especially for those samples taken from less aggressive diseases such as Myelodysplastic Syndrome (MDS) or chronic myeloid

leukemia (CML) [58]. To meet the need for better xenograft models, engineered organoid human bone may be implanted in murine models, providing a human bone-derived niche for stem cell engraftment and proliferation (Table 1). Works by Holzapfel et al., Reinisch et al., and Scotti et al. [61••, 63•, 64•] have yielded highly encouraging results, with evidence of long-term hematopoietic stem cells taking residence within the engineered human bone (Fig. 2).

While this represents a proof of principle in generating better xenograft models, further improvements and standardization of the models are needed. Future endeavors are likely to include coupling of engineering human bone with mice that are more human cell-compatible. One example is the use of NSG-SGM3 (or NSGS), a transgenic NSG strain that constitutively produces human cytokines SCF, GM-CSF, and IL3 into their serum [54•]. The NSG-SGM3 is superior in accepting and supporting the proliferation of human cells. Additionally, the appropriate cellular milieu for the establishment of a suitable hematopoietic niche needs to be identified. In particular, despite years of research and usage, human MSC sources are largely heterogenous and remain poorly defined. Good-quality and standardized MSC are requisite for engineering the human bone marrow niche reliably. Given the recent advantage in manipulation of embryonic stem (ES) [65] and iPS cells, these might provide a more reliable and reproducible source of MSC, for niche engineering. Finally, in reconstructing such a complex biological system as the bone marrow, bio-inspired engineering approaches based on developmental biology will be critical [66]. The process of cartilage remodeling in bone through endochondral ossification has been shown to be integral in the formation of a hematopoietic niche [67] and may be adapted to engineer a functional bone marrow, as demonstrated by Reinisch et al. [61••] and Scotti et al. [64•].

Bone Metastatic Disease

Metastasis is estimated to cause 90 % of cancer deaths [68], with the bone being a particularly common site of metastasis for cancers of the lung, breast, and prostate. Bone metastatic disease (BMD) is associated with high mortality and can also often be observed in cancers of the colon, stomach, bladder, uterus, rectum, thyroid, and kidney [69]. This is most pronounced in prostate cancer, which shows a peculiar predilection for bone, with 8 out of 10 advanced prostate cancer patients developing BMD [70]. BMD is also responsible for significant morbidity, resulting in bone pain, hypercalcemia, and pathological fractures [71].

Following escape from the primary tumor, cells are disseminated via hematogenous routes to distant tissues. It follows that premetastatic tissues provide cues to elicit cancer cell extravasation and migration into a metastatic-permissive environment. In bone metastasis, the process is believed to

Table 1 Summary of recent tissue engineering approaches in establishing humanized bone marrow as a hematopoietic niche

Matrix	Human cells loaded on matrix	Implantation method	Post-seeding culture time in vitro/in vivo	Human cells engrafted	Presence of primitive hematopoietic cells as defined by surface markers	Mouse strain	Reference(s)
Matrigel	Human BM MSC/ human peripheral blood endothelial cells	Injection	–/8–10 weeks	Cord blood (CB) mononuclear cells, leukemia cell line MOLM13 and Nalm6	Human CD45+	Nu/Nu or NOD-scid IL2rγnull (NOG)	Chen et al. 2012 [59]; Battula et al. 2013 [60]
Matrigel	Human BM MSC	Injection	–/8–12 weeks	Human CB CD34+	Human CD34+ CD38− CD90+ CD45RA− mouse LSK CD34− CD135− CD150+	NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG)	Reinisch et al., 2015 [61••]
Biphasic calcium phosphate particles	Human BM MSC	Surgery	1 week/8 weeks	Human CB CD34+; primary multiple myeloma (MM) cells	Human CD34+ or CD138+	RAG2−/−γc−/−	Groen et al. 2012 [62]
Type I collagen coated polyacrylamide hydrogen	Human BM MSC	Surgery	1–3days/4 weeks	Human BM CD34+ cells; leukemia cell line TF-1a	Human CD45+ mouse LSK cells	NOG	Lee et al. 2012 [5]
Calcium phosphate-coated polycaprolactone	Human BM MSC	Surgery	8 weeks/15 weeks	Human BM CD34+	CD45+ CD34+ (IHC) CD34+ 38− (flow)	NSG	Holzapfel et al. 2015 [63•]
Type I collagen meshes	Human BM MSC	Surgery	5 weeks/5–12 weeks	–	Mouse LSK CD34− CD135− CD150+	Nude	Scotti et al., 2013 [64•]

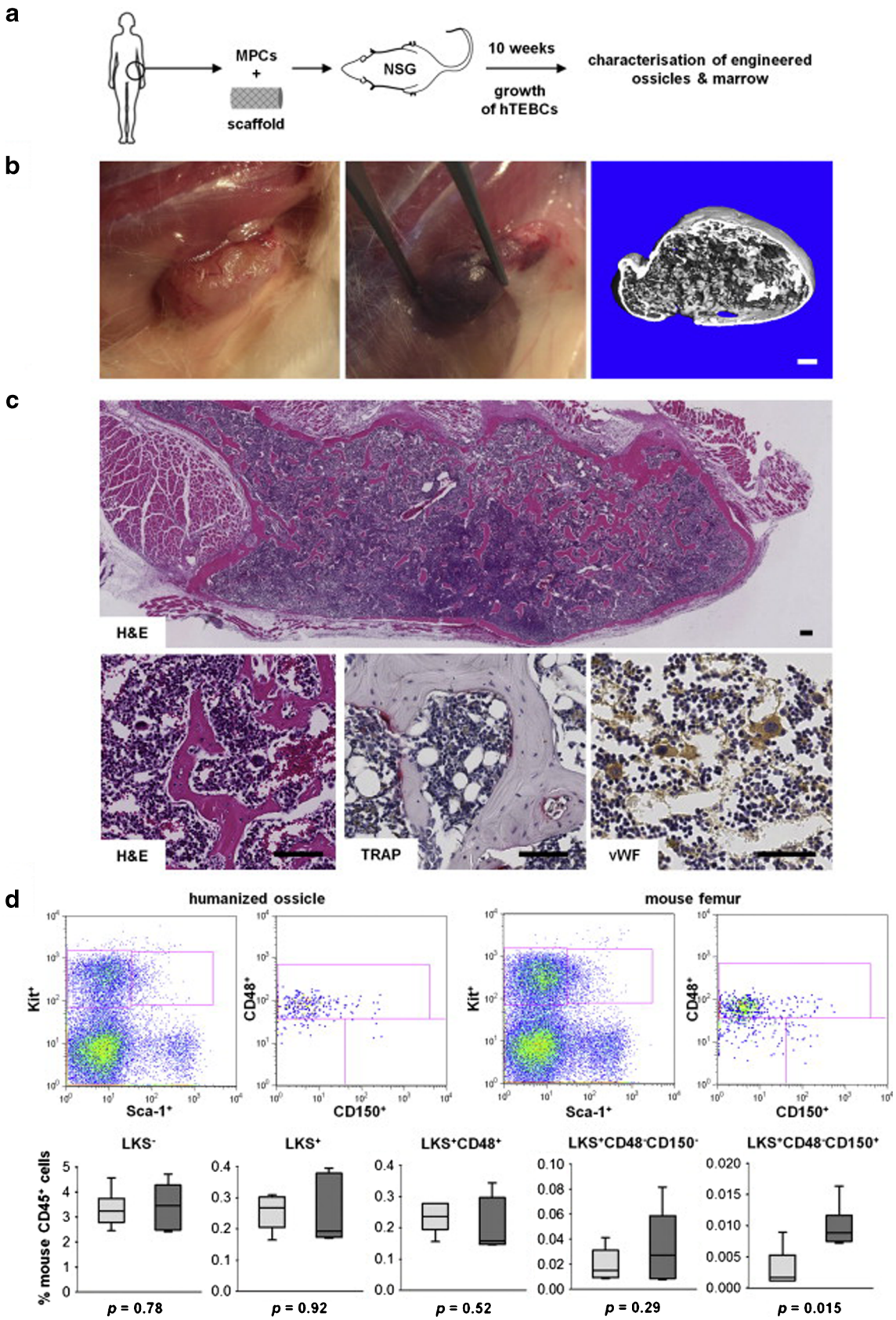

Fig. 2 Works by Holzapfel et al., Reinisch et al., and Scotti et al. [61••, 63•, 64•] have yielded highly encouraging results, with evidence of long-term hematopoietic stem cells taking residence within the engineered human bone

mirror the cytokine-mediated "homing" of hematopoietic stem cells to bone marrow [72] and bone-trophic prostate cancer cells have been shown to hijack hematopoietic stem cell niches within the trebaculae [73]. Following dissemination to these sites, significant cross-talks between the disseminated tumor cell (DTC) and the microenvironment continue to take place, leading to transformation into a "malignant niche" and activation of DTC towards a metastatically active phenotype. Much of these interactions are governed by osteoblasts, mesenchymal progenitors, and endothelial cells within the sinusoidal hematopoietic niche [49•, 74].

The role of bone marrow-derived MSC in BMD progression, in particular, has been the topic of much debate. Contrasting results have been demonstrated, for example for tumor-promoting [75, 76] and tumor-suppressive effects of MSC [77] in the microenvironment. Part of these differences may be explained by the phenotypic state of the MSC. Endochondral differentiation of MSC towards osteoblastic lineages is known to elicit chemoattractants, such as SDF-1 and MCP-1, and thus attract and retain B leukemia and metastatic breast cancer cells [78–80]. Once homed to bone marrow, tumor cells have been shown to interact with MSC or with their progeny (such as osteoblasts and adipocytes) to induce both pro-tumorigenic and inhibitory effects [81–83]. In light of such findings, it is clear that early crosstalk between disseminated tumor cells and the bone microenvironment is key to metastatic activation and disease progression [84], highlighting an urgent unmet need for adequate models of the disease.

Many experimental models of bone metastasis have been developed over the past century, which have been integral in identifying key molecular interactions in the bone metastatic niche. These models, however, are lacking in various aspects (Table 2). More recently, models combining features from the above have been employed that may provide more accurate representation of the clinical situation. Nemeth et al. first showed preferential homing of circulating prostate cancer cells towards subcutaneously implanted human fetal bone fragments over host skeleton in immunodeficient mice [95]. Subsequently, Moreau et al. found that co-implantation of engineered bone and tumor grafts also enables recapitulation of breast cancer metastatic events [96]. Besides increased accessibility to samples, tissue-engineered bone exhibits a major advantage in the ability to manipulate the engineered human bone prior to implantation [97]. For example, varying the stage of differentiation was found to influence extent of metastasis, suggesting a role of osteoblastic progenitors in homing [96]. Similarly, altering the state of the ECM may yield differences in metastatic responses. Tumor migration and proliferation, skeletal remodeling, and even molecular events [98] may then be interrogated longitudinally, aided by their extra-skeletal location and close proximity to skin [99]. The scaffolds used may also be modified to study the contributions of specific molecules in the process: modification of silk scaffolds with receptor activator of nuclear factor kappa-B ligand (RANKL), for example, increased breast cancer metastasis to subcutaneously implanted engineered bone constructs [100]. Additionally, the use of human cells facilitate the tracking of human-specific proteins, isolating graft-specific responses [101••]. Other advantages of this system include amenability to genetic manipulation prior to implantation and the use of human tissue to reduce confounders arising from xenogenic host cells. The use of tissue-engineered bone in a metastasis model thus allows the generation of a range of premetastatic conditions, followed by longitudinal and real-time assessment of critical cancer parameters. These include tumor size, extent of skeletal remodeling, and expression of osteogenic markers. Using such approaches, Holzapfel et al. demonstrated species-specific homing of metastatic prostate cancer cells to engineered human bone, where they contribute to significant osteolysis and cancer growth, recapitulating the clinical features of the disease [101••]. These studies and others suggest the utility of murine models with xenografted bone implants as humanized models of bone metastatic disease, providing unique opportunities to investigate poorly understood aspects of the disease [102•].

A major research theme in tissue engineering involves vascularization of implanted constructs, in order to promote tissue viability and engraftment. Establishment of a functional vascular network in xenografted bone is critical for effective

Table 2 Summary of existing models in the study of bone metastasis

	Experimental set up	Ref	Limitations
Two-dimensional (Petri dish cultures)	Cancer cells + ECM	[85]	Lack three-dimensional organization
	Mixed cellular co-cultures	[86]	
Three-dimensional (engineered tissue)	Cancer cells + ECM in a scaffold	[87–89]	Unable to recapitulate early metastatic events such as extravasation. Difficult to image
	Cellular co-cultures	[90]	
	Spheroidal cultures		
In vivo models	Subcutaneous implant of metastatic graft model	[91]	Unable to recapitulate human-specific responses
	Tibial injection of cancer cells	[92]	Technical challenges in imaging and studying early events
	Genetically engineered mouse models	[93]	
	Intracardiac injection of cancer cells	[94]	

models of bone metastasis, considering the roles of vascular transport and bone marrow endothelial cells in cancer cell transport and homing [103]. Several strategies have been developed to promote vascularization of engineered tissue following implantation, including biomaterial and scaffold modification, use of growth factors, and the use of co-culture systems [104]. The use of angiogenic cells in co-culture has also been explored, and endothelial lineages [26•, 105–108] have been incorporated into engineered tissue constructs to promote vessel formation. These angiogenic cells spontaneously form prevascular networks in vitro and anastomose rapidly following implantation, leading to perfusion of the construct. In experiments involving MSC-EPC grafts, the neovasculature formed within the graft is found to comprise cells of human origin, connected to host vasculature, suggesting concurrent vasculogenesis and angiogenesis [108]. It is anticipated that such efforts will have significant impact on refining the current xenograft models by reproducing the heterocellular composition of the bone marrow, which, in turn, are expected to have profound effects on cancer cell behavior [109, 110].

Primary Bone Malignancy

Bone-primary malignancies, including osteosarcoma (OS), are generally rare in occurrence, affecting less than 1 % of the general population in the USA [111]. OS may be categorized according to its location within the bone structures (intramedullary, cortex-associated) or disease entity (Paget's disease, fibrous dysplasia, Mazabraud's disease) [112]. A peculiar hallmark of the disease is the bimodal age distribution: OS affects mainly adolescents (10–14 years old) and older adults (>65 years old) [111, 113] and represents a significant health concern for these populations. However, the disease remains poorly understood, with severe limitations in existing diagnostic and treatment options [112]. A review of www.clinicaltrials.org (accurate to January 2016) revealed 349 clinical trials, of which 149 are completed (42.7 %); 145 are still recruiting, inviting, or active (41.5 %); 44 were terminated, withdrawn, or suspended (12.6 %); 10 are not yet recruiting (2.9 %); and only 1 approved for marketing (0.3 %). These statistics suggest extremely poor clinical translation of investigated techniques, highlighting the need for adequate disease models to investigate the molecular pathogenesis of OS.

Since 2011, there have been 5863 publications about OS, forming approximately 32.9 % of all publications to date (information from Web of Science, accurate to January 2016), demonstrating the increase in research prominence of OS over the last 5 years. Approximately 11.0 % of these publications were related to the use of animal models for studying OS, mostly involving murine models (6.4 % or 373 publications) or canine models contributed (4.6 %; 265 publications). It is interesting to note that dogs are predisposed to OS, leading to their utility as spontaneous OS models [114]. Canine models of OS have been identified to present parallel genetic features to human OS [115], including similarly dysregulated *p53, c-sis*, and *c-myc* profiles [116]. Crucially, they capture critical clinical features of the disease, such as diffuse pulmonary metastasis [117]. Using such models, Ranieri et al. correctly predicted the efficacy of a regime based on intra-arterial cisplatin and irradiation to achieve local tumor control and retain limb function [118, 119]. Canine studies are, however, largely prohibitive in terms of costs and scale, severely limiting their routine application in research studies. Moreover, significant differences remain between canine and human osteosarcomas [120].

In the murine models, human xenografts are introduced via inoculation of cells subcutaneously [121], direct injection into the tibia [122], or subperiosteal injection [123]. Thus generated, these models act as avatars to elucidate optimal drug regimes or as disease models for the evaluation of the role of oncogenic molecules in disease progression, including *p53* [121], IL-6/STAT3 [122], and ROCK1 [124]. Of these, orthotopic models are favored for better approximation to the osseous tumor microenvironment; multimodal positron emission tomography, computed tomography, magnetic resonance imaging, and bioluminescent imaging may be performed to achieve high-resolution imaging of tumor interactions with bone [125]. Technical challenges of such approaches remain, however, such as mortality arising from the intrabone injections [123], technically demanding imaging techniques and difficulty in harvesting or retrieving the xenografted cells.

As above, the use of tissue engineering approaches to generate bone (in this case, osteosarcomas) may provide similar value as an experimental model for osteosarcoma research. In fact, osteosarcoma lines such as MG62 and Saos-2 have historically been used in studies evaluating the compatibility of novel biomaterials with osteogenic cells [126, 127], providing early evidence of the technical feasibility of engineering an osteosarcoma in vitro. According to Villasante et al., an in vitro model of Ewing's sarcoma was found to be capable of replicating several features of the native bone tumor niche, including recovery of a hypoxic and glycolytic phenotype—features that are lost in monolayer cultures [128]. To such constructs, it has been suggested that co-cultures with human-derived mesenchymal stromal cells and/or endothelial cells may be introduced to mimic the cellular heterogeneity of the in vivo environment, further improving the biological fidelity of such models [129]. Corollary to this, such assembled human osteosarcoma-bone constructs may be implanted into immunocompromised mice, possessing sufficient biological complexity to capture the pathophysiological processes in tumor progression. This approach has, to our knowledge, not yet been attempted, and it remains to be seen if this model will provide a more clinically relevant model, particularly if malignant transformation and metastatic distribution can be faithfully reproduced.

Conclusions

Xenograft models are commonly used to establish the safety and efficacy of engineered tissue grafts prior to translation to clinics. More recently, such models have gained attention as experimental models of disease. Xenografted engineered bone tissues, in particular, have applications in models of leukemia, bone metastatic disease, and osteosarcoma. Major advantages of this approach include recapitulation of human-specific physiology including cancer cell homing and drug responses. Additionally, these models benefit from the extensive range of tools developed for evaluating both graft and host responses in preclinical tests. Significant challenges remain, however, which need to be addressed before such models may be reliably used. Graft viability is compromised by implantation, due to a range of issues including immunogenic responses to the xenograft and the lack of adequate vascularization. This is particularly pronounced in orthotopic, large animal models, which suffer from both immunorejection issues and compromised nutrient supply compounded by the size of constructs. As such, disease models remain largely limited to rodent models, in which research is currently being undertaken to improve the tolerability of the hosts and the biological performance of the grafts.

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Compliance with Ethical Standards

Conflict of Interest Chong Seow Khoon Mark, Bao Chaolemeng, Ng King Pan, Lim Jing, and Chan Kok Yen Jerry declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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