Advances in personalized treatment of metastatic spine disease

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Abstract: The spine is one of the most common sites of bony metastases, and its involvement leads to significant patient morbidity. Surgical management in these patients is aimed at improving quality of life and functional status throughout the course of the disease. Resection of metastases often leads to critical size bone defects, presenting a challenge to achieving adequate bone regeneration to fill the void. Current treatment options for repairing these defects are bone grafting and commercial bone cements; however, each has associated limitations. Additionally, tumor recurrence and tumor-induced bone loss make bone regeneration particularly difficult. Systemic therapeutic delivery, such as bisphosphonates, have become standard of care to combat bone loss despite unfavorable systemic side-effects and lack of local efficacy. Developments from tissue engineering have introduced novel materials with osteoinductive and osteoconductive properties which also act as structural support scaffolds for bone regeneration. These new materials can also act as a therapeutic reservoir to sustainably release drugs locally as an alternative to systemic therapy. In this review, we outline recent advancements in tissue engineering and the role of translational research in developing implants that can fully repair bone defects while also delivering local therapeutics to curb tumor recurrence and improve patient quality of life.

Keywords: 3D printing; biomaterials; drug delivery; metastasis; spine surgery

Introduction

The spine is one of the most common sites of bony metastases (1), where cadaveric studies have shown that 30–90% of patients diagnosed with cancer will have spinal metastases by time of death (2). Metastatic spine tumors commonly originate from solid tumors of the lung (3), prostate (4), breast (5) and non-solid hematological sources. Due to its vital role in providing postural support, movement, and protection of the spinal cord and nerve roots, metastatic spine disease often leads to significant morbidity in patients. Disruption of normal bone turnover by local osteogenic or osteolytic effects of metastatic tumors creates a detrimental instability in the weight bearing

spinal column. This in turn can lead to pain, pathological fractures, and ultimately neurological deficits through involvement of adjacent neurovascular structures (6). Advancements in management of patients with metastatic disease has led to increased survivability over the past decades, making effective management of spinal metastases critical to improving their quality of life throughout the course of their illness (7-9).

Current modalities for management of metastatic spine disease include radiotherapy, surgery and systemic chemo/antiresorptive therapy (10). However, surgery has proved to be the most effective intervention in patients with neurological deficits and bony instability (11,12). In 2005, Patchell *et al.* (11) published a landmark article that

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solidified the role of surgery in the management of spinal metastasis. In this study, patients with metastatic epidural spinal cord compression were randomized to undergo surgical decompression in addition to radiation therapy or radiation therapy alone. The study was terminated early as surgery together with radiation therapy was shown to be superior to radiation therapy alone in the interim analysis. Regarding spine instability, the development of the Spinal Instability Neoplastic Score (SINS) in 2010 has helped define spinal instability in a reproducible manner and to guide surgical decision making (12-14).

The basis of surgical interventions in metastatic spine disease involves removing or debulking a tumor to alleviate its biochemical and mass effects on the surrounding bony and neurovascular tissue. This can be followed by instrumented fusion of adjacent segments to restore structural integrity to the spinal column (15). Removal of the tumor can lead to unstable critical size defects that limit natural bone remodelling, requiring use of bone substitutes to aid healing (16). This is not without risk, as more than 10% of these patients must be re-operated on often due to hardware failure or other complications (17,18), which can become a costly burden on the healthcare system (19). Additionally, tumor recurrence and continuous local bone loss require systemic chemo- and antiresorptive therapy which can cause significant systemic side-effects such as osteonecrosis of the jaw (20) or renal toxicity (21), limiting their prolonged use. Recent trends have demonstrated the potential of novel bone substitute materials for delivering therapeutics locally to avoid side-effects associated with systemic therapy while at the same time promoting bone regeneration. In summary, there is still a high reoperation rate with many being secondary to local recurrence (18). An understanding of the cellular and molecular basis of the metastatic process might help improve local control following spinal surgery.

Cellular and molecular basis of bone metastasis

Seed and soil hypothesis

While there are still significant questions about metastasis that have yet to be answered, the discoveries that will be discussed have led to changes in therapeutic approaches that have had a significant impact on patients' outcomes. Currently, "the "seed and soil" hypothesis" on distant metastasis emphasises the importance of the interplay between both the tumor cells and target organ characteristics in the success of the metastatic process (22). This hypothesis, described by Dr. Paget in 1889 (22,23), posits that metastatic cells travel far from the primary site, bury themselves in the target organ, such as bone marrow, and lay dormant until certain conditions drive the cells to proliferate. This was based on observations that rates of metastases to different organs did not correlate with the relative share of the blood flow based on the autopsy reports of 735 patients with breast cancer (23). Paget also contrasted his findings in patients with breast cancer to those with other primary tumors. These observations were later confirmed by Hart et al. (24) in a metastatic melanoma rat model. Tissues from different organs (lung, kidney and ovary) were ectopically implanted in animal hind limbs, and a metastatic melanoma model was established by intravenous injection of tumor cells. The authors noted that melanoma tends to preferentially metastasize to pulmonary tissue and concluded that the process of metastases is not random. Studies have also shown that metastases can exert a selective pressure on tumor cells, thus resulting in metastatic tumors that differ from the original tumor (22,25-27). In 1970, Fidler showed that only 1.5% of melanoma tumor cells which enter the circulation will survive beyond 24 hours (25). Furthermore, Fidler et al. (27) showed that metastatic tumors are monoclonal in nature. In these experiments, 2 different melanoma cell lines were injected intravenously as either a homogenous or a heterogenous mixture. The resultant lung metastases were all found to have originated from a single cell line suggesting that only certain tumor cells can successfully metastasize (27). These findings could help explain why metastatic tumors might respond differently than the parent tumor when exposed to the same therapeutic agents.

Tumor cells that manage to survive and reach the skeleton must interact with a microenvironment unique from other organs. Furthermore, bone itself can be subclassified into cortical and cancellous, both of which differ in physical structure and metabolic activity. The bone, therefore, offers metastatic cells unique micro-environments that are termed "niches" (28). The impact of the physical characteristics of the micro-environment on tumor cell behavior was studied by Ruppender et al. (28,29). Using 2D models that simulated tissues with different structural rigidity, they were able to show that tumor cells expressed increasing levels of parathyroid hormone-related protein (PTHrP) with increasing rigidity of their physical environment (29). The rigidity in this test model was achieved through

seeding cells on polyurethane (PUR) films that were under varying degrees of tensile stress (29). The gene responsible for PTHrP expression is GLI2, which is a zinc finger transcription factor which increases in expression with increased environmental stiffness (30-32). PTHrP/GLI2 may be a potential therapeutic target against skeletal-related complications of bone metastases. This was demonstrated by Gallwitz *et al.* (33) whereby inhibition of GLI2 transcription through guanine-nucleotide analog (6-Thioguanine) decreased PTHrP expression, PTHrP-induced osteolysis and hypercalcemia in a mouse metastasis model. These findings are significant given the fact that PTHrP is known to increase osteoclastic activity and bone resorption. Bone is also a dynamic structure that is being constantly remodeled in a process that couples bone resorption and bone formation (28). Bone resorption is carried out by osteoclasts while osteoblasts form new bone during the remodelling process (28). Bone remodelling centers tend to be rich in nutrients and growth factors compared to regions that are not undergoing remodeling. Tumor cells that metastasize to remodelling zones tend to have higher growth potential compared to those that metastasize to bone that is quiescent (28). Conditions and medications that increase bone turnover have been shown to increase metastatic tumor growth within the skeleton (28,34,35).

Stages of metastasis

The process of bone metastasis can be grouped into four stages: colonization, dormancy, reactivation and growth (28). Each of these stages varies based on the type of tumor cell and the type of niche where it arises (28). Colonization is the stage in which metastatic tumor cells enter the bone marrow (28). Studies have shown that colonization of the skeleton does not necessarily lead to overt metastatic boney lesions (28,36). Domschke studied the impact of finding disseminated tumor cells within a bone marrow biopsy in a cohort of 1,378 breast cancer patients (36). Of these, 621 patients had positive bone marrow biopsies, but only 139 (22.4%) ended up developing boney metastasis (36). The next step is dormancy in which tumor cells start to adapt to their new environment (28). For example, dormant multiple myeloma cells are more likely to be present in niches rich in osteoblastic cells (37). Another study showed that dormant multiple myeloma cells were resistant to melphalan, which is an alkylating agent used to treat multiple myeloma (38). These findings could help explain why patients can undergo early and late relapse following treatment. Tumor cells which are dormant can be reactivated by a variety of stimuli (28). While the exact mechanisms of reactivation are still being investigated, it is believed that osteoclasts play an important role by changing the biophysical environment within a niche and/or altering cell signalling pathways (28,38). Once released from dormancy, tumor cells form micro-metastases which then modify the local environment through cell signalling and ultimately result in overt metastasis (28).

Antiresorptive/immunotherapy in spine metastasis

Given the role that osteoclasts play in the reactivation and growth of tumor cells, medications which reduce osteoclastic activity can help control the progression of skeletal metastasis (28). A randomized control trial published in 2015 showed that adjuvant denosumab therapy in postmenopausal women with breast cancer reduced the number of pathologic fractures and delayed the time to the first clinical fracture (39). Another randomized control trial published by Saad *et al.* (40) showed that zoledronic acid reduces the risk of developing pathologic fractures in patients with hormone-refractory metastatic prostate carcinoma. A 2015 meta-analysis of the results of randomized control trials on adjuvant bisphosphonate therapy in breast cancer showed that bisphosphonates reduce the risk of cancer recurrence in the bone (41). Very recently, a systematic review indicated that use of bisphosphonates in management of metastatic disease to the bone was cost effective and resulted in lower mortality and improved quality of life for patients (42). In contrast, use of denosumab was found to be "marginally more effective" for improving outcomes than bisphosphonates (42). However, the high price of the drug resulted in a much higher cost for each quality-adjusted life year gained as compared to bisphosphonates, rendering denosumab treatment not cost effective (42).

Local vs. systemic treatment

Bisphosphonates and denosumab are both administered systemically, which has been associated with several negative side effects. High systemic doses of zoledronic acid were found to be associated with renal function deterioration (40). Other potential side effects include fevers, myalgia, hypocalcaemia, osteonecrosis of the jaw and atrial fibrillation (43). To circumvent these challenges, efforts

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are being made to explore effects of local bisphosphonate delivery at the site of boney metastases. We have previously assessed the efficacy of local *vs.* systemic delivery of zoledronic acid in a metastatic murine xenograft model (44). A metastatic tumor was established in the proximal tibia and each animal was treated with a weight adjusted dose of 0.025 mg/kg of zoledronic acid once a week delivered either systemically or locally. The animals were treated for a total of 4 weeks. We showed that mice which received locally administered zoledronate had a statistically significant 44.8% increase in bone volume/tissue volume % relative to those receiving systemic zoledronate (44). These results show that local delivery of zoledronate can improve local bone quality in the setting of bone metastasis. We also found that there was an increase in tumor cell apoptosis and a decrease in tumor cell proliferation, but neither of these findings reached statistical significance (44). While these results are impressive, they are likely to be challenging to implement in a clinical setting given the resources that would be needed to perform the weekly procedures. Therefore, development of drug delivery devices may be a good approach to local delivery of bisphosphonates or other agents such as denosumab antibodies.

Current advancements in tissue engineering and targeted drug delivery

Development of an optimal solution to address the shortcomings of current surgical management of patients with metastatic spine disease will require a multidisciplinary approach. As outlined earlier, a major focus for management of these patients is adequate viability of the graft used to fill the defect created after resection of a metastatic lesion from the vertebrae. Current treatment options have a limited ability in preventing tumor recurrence, promoting bone regeneration, and restoring the original structural integrity of the involved segments. Outlined below are recent biomedical advancements that explore novel solutions to limitations in surgical management of patients with metastatic spine disease.

Bone cements

Bone cement has been used extensively in surgery for more than half a century, with the first orthopedic application being performed by the English surgeon Dr. John Charnley for implant fixation in hip replacement operations (45). Use of cements in surgical management of metastatic spine

disease allows for restoration of structural integrity, leading to an improved pain score and function in patients (46-49). Commonly used cements include calcium phosphate and polymethyl methacrylate (PMMA), with a variety of composite formulations with other compounds existing to achieve unique structural and chemical properties.

Calcium phosphate cement (CPC) is bio-resorbable (50,51), which can provide a scaffold within a defect for eventual bone regeneration. In its pure form, however, CPC has poor stress tolerance and is brittle, making it unsuitable for use in the weight-bearing spine (52,53). However, composite formulations of CPC have more favorable mechanical properties, as outlined by Hu *et al.* (54) in a recent study with the use of silk fibroin to reinforce the cement. Although CPC alone is a poor modality for sustained drug release (55), a very recent report demonstrated that conjugating CPC with polylactic-co-glycolic acid (PLGA) microspheres allows the compound to release 25% of loaded Alendronate over a 148-day period (55). Another study has shown that zoledronate impregnated calcium deficient apatite (CDA) was able to sustainably release the drug to inhibit osteoclast number by 85% and decrease osteoclastic bone resorption by 3.3-fold without hindering osteoblast function in an *in vitro* rabbit bone culture (56). Despite its limited use as a standalone bone substitute in the spine, CPC and its composite formulations carry high potential for sustained local delivery of therapeutics in spinal metastasis patients.

PMMA cement is widely used in vertebroplasty procedures. The ability of PMMA to create a mechanically stiff core inside the vertebrae make it ideal for filling structural voids within the spine (57). Unique antitumor properties of PMMA cement have also been proposed, with heat induced tumor necrosis from the high temperatures of cement curing (58), to direct cytotoxic effects of PMMA monomers on cells in proximity to the cement (59). Recent trends in drug therapy have also shown promising potential for PMMA as a tool for local drug delivery. Antibiotic impregnated PMMA cement is widely used in surgical procedures to provide sustained and local concentrations of a multitude of antibiotics, such as tobramycin, while minimizing systemic exposure to these drugs (60). Interestingly, a recent study investigated local delivery of zoledronate to treat bony malignant tumors (61). In this study, zoledronate was loaded into commercially available formulations of hydroxyapatite and polymethyl methacrylate (PMMA) bone cement. The cement containing zoledronate was found to decrease tumor cell viability. Unfortunately,

these effects were not sustained over the 14-day course of the experiment (61). The authors also noted that although the zoledronate-hydroxyapatite combination did exhibit antitumor effects, these effects were weaker compared to the zoledronate-PMMA formulation. This difference was hypothesized to be due to higher affinity of zoledronate to hydroxyapatite (61). This study also assessed the impact of local zoledronate delivery on serum creatinine and blood urea nitrogen as surrogate measures of renal function, and all parameters remained within normal limits (61). While these implants were able to produce an antitumor effect, it was only sustained for 14 days (61). Given what we know about the process of bone metastasis, and the presence of dormant tumor cells within the bone, such a short duration is unlikely to be of a large clinical benefit and more research into this topic is warranted. Indeed, one group recently reported in a phase-1 clinical trial of 17 patients that local delivery of zoledronate through bone cement was safe, did not cause any side effects and may have reduced local recurrence of giant cell tumor of bone (62). It is important to note that a phase 2 randomized control clinical trial is currently underway by this same group at St. Louis University investigating whether 4 mg zoledronate mixed with PMMA cement can decrease the local recurrence rate of giant cell tumor of bone following curettage in 120 patients. All these reports indicate that local delivery of bisphosphonates within a structural carrier has high potential for blocking spine metastasis recurrence and stabilizing the bone following resection.
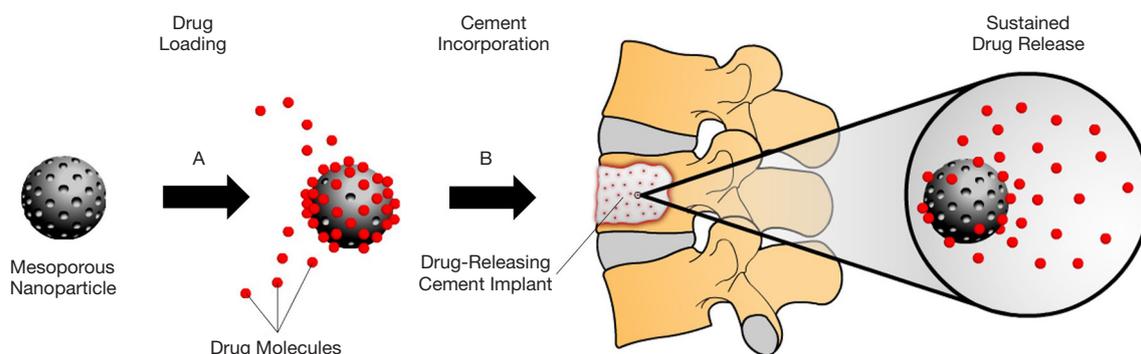
Nanoparticles

Nanotechnology has emerged over the past decades as a promising approach for targeted drug therapy. Nanoparticles (NP) allow for therapeutic control in dimensions never seen before in modern medicine (63). These versatile particles posses many different chemical and biophysical properties, making them very attractive for localized drug therapy in a multitude of diseases. They can function as drug sequestrants, prolonging the half life of drugs by protecting their degradation and elimination from the body (64). Through a phenomenon known as "Enhanced Permeability and Retention (EPR)" outlined by Matsumura *et al*. (65) in 1986, nanoparticles have been observed to passively target and accumulate in malignant tissue due to the increased permeability of their hypervascular environment and decreased lymphatic drainage. Additionally, preclinical animal studies of pH-responsive nanoparticles loaded with chemotherapeutics have demonstrated increased drug activity within acidic tumor environments when compared to pH-unresponsive nanoparticles or free drug administration (66,67).

Nanoparticles have been studied extensively as a viable option for sustained and local delivery of chemotherapeutics and antiresorptive medication, making them good candidates for study in metastatic spine disease. Doxorubicin-conjugated polyethylene glycol (PEG) nanoparticles have been investigated for intravenous treatment of primary and metastatic human osteosarcoma cell lines (68). This *in vitro* study demonstrated that the nanoparticle-conjugated Doxorubicin achieved the same levels of tumor cellular uptake at one tenth of the concentration of free Doxorubicin. This translated to a 40% greater inhibition of tumor growth when compared to systemic delivery of free drug in a mouse tumor model (68). Another study demonstrated the effectiveness of intra-tumoral delivery of Paclitaxel-conjugated hyaluronan nanoparticles in treatment of breast cancer cell lines (69), where the nanoparticle-conjugated Paclitaxel was able to achieve the same therapeutic effect as free drug. However, the *in vivo* intra-tumoral injection of nanoparticle-conjugated Paclitaxel surprisingly led to a 50% decrease in tumor size over 57 days as compared to an almost 5-fold increase in size in the free drug intratumor injection group (69). Furthermore, mesoporous silica nanoparticles in combination with PMMA cement are being studied for use in targeted and sustained drug delivery (70-72). Incorporation of drug-loaded nanoparticles into cement scaffolds filling large bone defects can have applications for surgical management of metastatic spine disease (*Figure 1*). These studies have showcased the superior ability of nanoparticles in extending and concentrating the therapeutic actions of drugs compared to treatment with free drug which is the standard of care in cancer therapy today.

3D printing

With the advent of three-dimensional (3D) printing technology, also known as additive manufacturing, it has become possible to design and materialize complex objects without the need for sophisticated manufacturing equipment. With the aid of user-friendly computer design software, researchers can obtain and test any desired structure in a timely and cost-effective manner (73). A diverse variety of materials are possible to 3D print, including metals, ceramics, polymers, and hydrogels

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Figure 1 Drug-loaded mesoporous nanoparticles for localized drug delivery using bone cement in metastatic spine disease. (A) Nanoparticle loaded with a desired drug. Successful binding to drugs with different chemical properties can be achieved through altering the nanoparticle composition or its surface properties. (B) Drug-loaded nanoparticles are mixed with bone cement. The nanoparticle-containing cement is then used to fill a critical size bone defect in the spine. After cement hardening, drug-loaded nanoparticles dispersed within the cement will slowly release drugs into surrounding tissue in a sustained manner.

containing live cells for bioprinting. Some of the polymers used are FDA approved, such as polylactic acid, polycaprolactone and polyglycolic acid, which also can be designed to possess appropriate mechanical properties for orthopaedic applications.

The use of biocompatible and osteoinductive compounds in 3D printing has paved the way for novel approaches to bone regeneration and tissue engineering. A recent study by Heo *et al.* (74) demonstrated feasibility of coating osteoinductive fish bone extract on polycaprolactone 3D-printed scaffolds in an *in vitro* osteogenic model using a mouse pre-osteoblast cell line. Their results demonstrated that this treated construct increased calcium deposition onto the scaffold by more than 5-fold (74). A study by Hutmacher *et al.* (75) concluded that PCL 3D-printed scaffolds allow for continued proliferation and matrix production of human fibroblasts and periosteal cells over a 4-week period in an *in vitro* model. An *in vivo* sheep tibia model by Cipitria *et al.* (76) demonstrated that 3D-printed PCL scaffolds allow for retention and prolonging the effect of the growth factor bone morphogenetic protein (BMP), decreasing the need for administration of costly supraphysiological doses of the protein. These studies provide new insight into the versatility of 3D-printed biocompatible constructs in providing a scaffold for bone regeneration. Several studies have indicated feasibility of using 3D-printed ceramics as maxillofacial bone substitutes (77-79). These types of scaffolds could also presumably be used as drug delivery devices.

In addition to tissue regeneration and repair, 3D-printed

scaffolds have been shown to be effective at delivering drugs locally in a sustainable manner. Our laboratory has recently demonstrated sustained Doxorubicin delivery in an *in vitro* 2D prostate cancer model using a novel nanoporous PORO-LAY 3D-printed scaffold (80). The PORO-LAY polymer is a thermoplastic polyurethane (TPU) and polyvinyl alcohol (PVA) co-polymer (81). This polymer is unique in that it can be 3D-printed into any desired shape as a rigid plastic. However, the PVA component dissolves upon washing the construct with water, transforming it into a sponge riddled with drug-absorbent nanopores (80). We demonstrated that doxorubicin delivery from the nanoporous scaffold was able to achieve roughly 60% reduction in metabolic activity of patient-derived prostate cancer spine metastasis cells. This was comparable to the same reductions in metabolic activity observed with direct treatment of the cells with Doxorubicin (80). A follow up study testing the effectiveness of these scaffolds for targeted delivery of bisphosphonates is currently underway.

Bioprinting

Three-dimensional printing has revolutionized our capacity for creating physical objects, and the same principle has great potential for creating complex tissue-like structures composed of living cells and other biomaterials that closely mimic *in vivo* microenvironments. Advancing technology, free access to design software and reduction in overall cost of bioprinter hardware has allowed for creation of sophisticated machines that can handle and seed cells in a

safe and precise manner (82-85). There are several types of bioprinting techniques, but the most widely used involves suspending cells inside an extracellular matrix-like bioink material and extruding layers one on top of the next. Natural bioinks commonly consist of collagen (86), alginate (87), chitosan (88), and silk fibroin (89). Synthetic bioinks consist of PCL (90), polyethylene glycol (PEG) (91), and hydroxyapatite (92), which are suitable for bioprinting more rigid models for studying cartilage and bone (91). Current applications of bioprinting involve creating testing models for various tissue types such as skin (93), cardiovascular (94-96) and bone (97). Regarding bone, bioprinting is effectively combined with 3D printing to create a rigid scaffold with subsequent seeding of osteoprogenitor cells within the scaffold. Addition of growth factors or defined osteogenic medium into the bioink will provide additional stimulation for progenitor cells to undergo osteogenic differentiation within the construct (98).

Bioprinting can be an effective tool for studying metastatic spine disease through creation of tumor models that more accurately study tumor behavior in a three-dimensional tissue-like environment as opposed to current 2D cultures (85,99). Cells behave and respond to therapeutics differently in a 3D environment as opposed to the 2D environment of conventional cell culture techniques (100,101). One area of interest is drug sensitivity of tumors when they are arranged as 3D spheroids. A study by Zhao *et al.* (100) showcased how tumor spheroids exhibited greater resistance to chemotherapeutics than 2D models. The presence of stromal cells in the microenvironment can greatly influence tumor growth and progression. A study by Zhou *et al.* studied the effects of co-culturing human breast cancer cells and osteoblasts in a bioprinted 3D model. They observed increased breast cancer proliferation and a reduction in proliferation of osteoblast cells. Additionally, breast cancer cell secretion of vascular endothelial growth factor (VEGF) was increased while alkaline phosphatase (ALP) secretion by osteoblast cells was decreased. Bioprinting provided the advantage for precise placement of cells in separate, specific compartments that allowed for cell-cell communication and analysis of cell proliferation in three-dimensional space (101). Hence, bioprinting can allow for creation of complex three-dimensional tumor models that mimic the *in vivo* bone environment. This provides more clinically relevant testing of therapeutics and cell-cell interactions, serving as an offshoot for subsequent animal studies.

Animal models

Animal studies are an important initial step in assessing the clinical applicability of *in vitro* studies. Promising *in vitro* results must be confirmed within an *in vivo* environment to account for variables such as toxicity, immune response and pharmacokinetics. Animal models have been extensively studied with tissue-engineered constructs that promote *in vivo* bone regeneration (102-104). These constructs can be a combination of 3D-printed scaffolds, osteoprogenitor cells and growth factors (103,105-107). Common osteoconductive materials used in these scaffolds are hydroxyapatite, PCL, coral and ceramics, which contain a nanoporous structure ideal for host cell invasion (102,108-112). Additionally, different animal species provide certain advantages for studying bone regeneration. Smaller animals, such as mice, are suitable for studying ectopic bone formation (105). Due to their more similar size and mechanical loading characteristics as compared to humans, large animals, such as pigs and sheep, are suitable for studying tissue regeneration within bone defects through utilization of engineered constructs (105).

Large constructs designed to fill critical size bone defects are limited in their capability for widespread bone regeneration due to inadequate angiogenesis throughout their structure (113). Sathy *et al.* (102) used a multilayered construct design with alternating layers of osteoconductive PCL and calcium phosphate ceramic with angiogenic collagen/fibronectin zones. These zones allowed for through-the-thickness vessel formation inside the scaffold, resulting in widespread tissue regeneration inside the construct in a mouse model. Another approach for scaffold vascularization is providing an axial blood supply by incorporating the scaffold around an existing blood vessel (103,104,114). A study by Zimmerer *et al.* (104) vascularized a hollow beta-tricalcium phosphate scaffold using the thoracodorsal trunk of sheep. They observed that over a 6-month period, the scaffold had transformed into a solid bioartificial bone graft with widespread vascularization. Another study by Kaempfen *et al.* (113) vascularized decellularized trabecular bone cylinders with an axial blood supply from a branch of the axillary artery in rabbits. They compared widespread vascularization and bone formation between scaffolds that were ectopically incubated for 6 weeks before implantation into a segmental humerus defect and vascularized scaffolds that were implanted without

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incubation. Their results showed more vascularization throughout the incubated implant compared to implantation without prior incubation (113). However, the degree of bone formation they observed in both implants was minimal which they attributed to local inflammation around the bone defect.

In addition to these *in vivo* studies that showcase the ability of tissue-engineered constructs for bone repair, several *in vivo* human xenograft (115) and, more recently, patient-derived xenograft animal models (116) of various cancer types exist. The premise of these models is to implant human cancer cells from established/characterized cell lines or patient-derived tumor cells either subcutaneously or in the bone of these animals for example. Next, the animals can be treated with novel systemic therapeutics, nanoparticle carriers or implantable constructs following tumor resection. Most recently, efforts to model the human immune response to cancer have been made by "humanizing" immunocompromised animals through implantation of human immune cells within their bone marrow. A study by Shafiee *et al.* (117) humanized immunodeficient mice through inoculation of their bone marrow with human CD34+ cells. Upon xenografting human breast cancer cells, they observed that the humanized mice showed less tumor burden and metastasis compared to immunodeficient mice. These types of animal models can allow for addressing feasibility and optimal dosage requirements for novel therapeutics and carriers of therapeutics by creating animal disease models that more closely resemble human tissue.

Several studies have successfully shown how intravertebral tumor models can be used reliably to study neurological deterioration in animals (118-122). A study by Tatsui *et al.* (118) demonstrated how L-3 vertebral human lung cancer xenografts in mice can lead to paraplegia over 30 days. Their histological findings highly correlated with motor function assessment of these mice over the course of the study (118). Studies such as this are highly suitable for modelling spine metastasis. However, no studies to date have used such a spine metastasis model to study therapeutic interventions as most models focus on long bones of animals (44). Nonetheless, resection in the mouse/rat spine followed by implantation with bone substitutes or 3D printed constructs will be far more difficult than in the long bone of these animals. Although more costly, rabbits or larger animals may provide a larger anatomical site (123) in which to perform resection of induced tumors. This will be more feasible for implantation of biomaterials for bone repair and anti-cancer treatment. However, generating

immune deficient rabbits or other large animals will be costly and not mainstream, which limits their potential xenograft studies (124). Advancing the rodent spine metastasis models outlined in this review is likely the most cost-effective way to study tissue engineering strategies to treat spine metastasis.

Future directions

Despite the exciting advancements in the fields of targeted drug therapy and tissue engineering to treat and repair resected spinal metastases, limited human trial data are available to assess the true clinical applicability of these innovations. Personalized treatment of resected metastatic spine tumors using 3D printing technology appears most promising. Indeed, an ongoing clinical trial at Southern Medical University in China is applying 3D-printed implants to bone defects during metastatic spine disease treatment (125). Amazingly, this trial is a multicentered randomized controlled study including 300 participants, which is scheduled to be completed by December of 2021 (125). If 3D-printed constructs as a bone substitute are found to be a valid solution, this may prompt further consideration by regulatory bodies such as the FDA. These new insights further support the idea of using 3D printing in personalized treatment for spine metastases in the near future.

Conclusions

Improved quality of life continues to be an important goal for managing patients with metastatic spine disease. The studies outlined in this review have demonstrated how tissue engineering is being used for bone repair and regeneration. Tissue-engineered bone substitutes can also circumvent the limitations associated with bone grafting, such as donor site complications and limited supply. Furthermore, these novel bone substitutes can stabilize large bone defects and deliver chemotherapeutics locally to inhibit cancer recurrence and minimize toxicity associated with systemic drug delivery. Many of the bone substitutes outlined in this review possess capacity to deliver therapeutics, with antibiotic impregnated PMMA being the most clinically relevant. By combining recent advancements in tissue engineering and targeted therapy, new approaches to stabilize large bone defects, promote bone regeneration and locally deliver a specific cocktail of therapeutics is within reach (*Figure 2*). There is a great opportunity to achieve this goal; however,
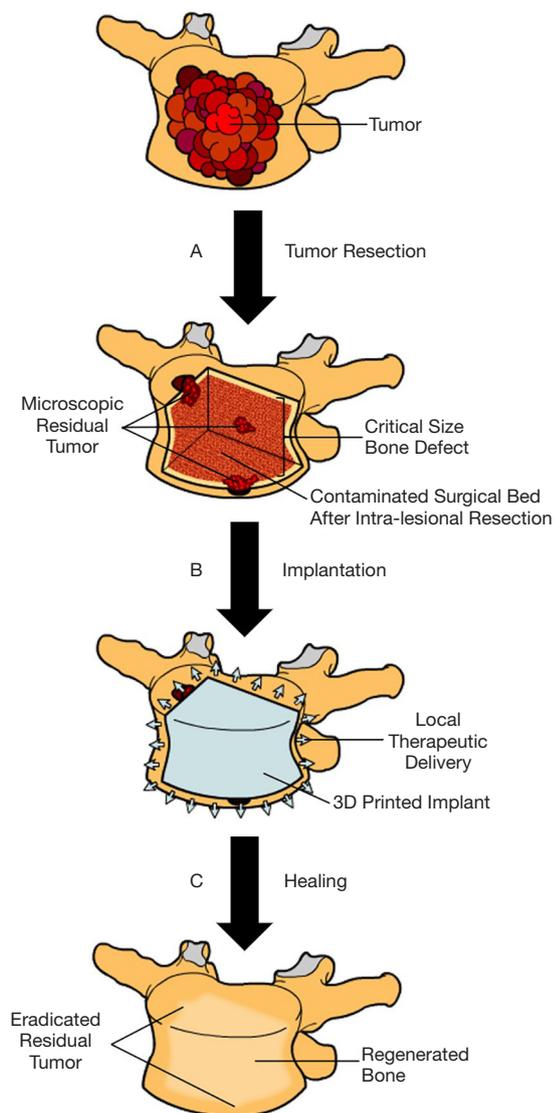
Figure 2 A novel, personalized therapeutic approach using a tissue-engineered drug releasing 3D implant to fill a critical-sized bone defect and promote bone regeneration. (A) Tumor is surgically resected from bone leaving a large defect with residual tumor. (B) A patient-specific 3D-printed osteoinductive/osteoconductive implant that is loaded with therapeutics is implanted inside the defect. The implant will continue to release the therapeutic locally after surgery. (C) Continuous local therapeutic delivery will prevent cancer recurrence within the vertebra while promoting regeneration of bone within the implant.

multidisciplinary strategies combining basic science, engineering and clinical principles must continue to be applied in future work.

Footnote

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