Tissue-engineered models of human tumors for cancer research

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Abstract

**Introduction**—Drug toxicity often goes undetected until clinical trials, which are the most costly and dangerous phase of drug development. Both the cultures of human cells and animal studies have limitations that cannot be overcome by incremental improvements in drug-testing protocols. A new generation of bioengineered tumors is now emerging in response to these limitations, with potential to transform drug screening by providing predictive models of tumors within their tissue context, for studies of drug safety and efficacy. An area that could greatly benefit from these models is cancer research.

**Areas covered**—In this review, the authors first describe the engineered tumor systems, using Ewing's sarcoma as an example of human tumor that cannot be predictably studied in cell culture and animal models. Then, they discuss the importance of the tissue context for cancer progression and outline the biomimetic principles for engineering human tumors. Finally, they discuss the utility of bioengineered tumor models for cancer research and address the challenges in modeling human tumors for use in drug discovery and testing.

**Expert opinion**—While tissue models are just emerging as a new tool for cancer drug discovery, they are already demonstrating potential for recapitulating, \textit{in vitro}, the native behavior of human tumors. Still, numerous challenges need to be addressed before we can have platforms with a predictive power appropriate for the pharmaceutical industry. Some of the key needs include the incorporation of the vascular compartment, immune system components, and mechanical signals that regulate tumor development and function.

**Keywords**

bioengineered tumors; biomimetics; bone tumors; drug discovery; microenvironment; tissue engineering; vascularization

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1. Introduction

Historically, cancer has been studied in two dimensional (2D) monolayer cultures, small animal models, and samples of human tumors [1]. When cultured in vitro, cancer cells lose many of their in vivo features, because of the lack of environmental signals present in native tumors [2]. In 2D culture, cells are deprived of the tissue matrix that is known to regulate tumor progression. Indeed, the lack of cell matrix interactions that are involved in native tumors leads to changes in cell phenotypes and gene expression. As a result, some important aspects of tumor biology – most notably angiogenesis and metastasis – cannot be properly assessed in monolayer culture. Animal models also have limitations, as they often fail to represent the pathology of human tumors [3-6].

In principle, the ability of in vitro assays and animal models to provide clinically relevant information is essential for drug development. Today, eight out of nine drugs that are successfully tested in animal models or monolayer cultures of human cells fail at some stage of clinical testing in patients [7-10]. One of the key challenges in cancer research is to develop predictive in vitro models of human tumors – primary and metastatic – for identification of therapeutic targets and drug testing. Bioengineering methods that have transformed stem cell research and application of stem cells in regenerative medicine are just starting to enter the field of cancer research to meet this critical need. At this time, simple culture formats, such as tumor spheroids, cancer cells in scaffolds and small cancer organoids, are being complemented by bioengineered tumors providing cancer cells with a tissue context incorporating the extracellular matrix (ECM), stromal cells and physical signals [1,2,5].

Tissue engineered tumor models have been developed to recapitulate some features of the tumor environment while enabling control of environmental factors and measurement of cell responses. We have recently used the bioengineered human bone as a niche for Ewing’s sarcoma cells to build a 3D tissue model of this tumor. We demonstrated that a number of genes related to focal adhesion and cancer pathways that are expressed in the native tumor are down regulated in monolayer cultures of tumor cell lines (Figure 1) and re expressed when the same cells are cultured within a tissue engineered bone [11].

In this commentary, we reflect on the state of the art in 3D tumor modeling and tissue engineered tumor systems. Although these models are just emerging as a new tool for cancer drug discovery, they are already demonstrating potential for recapitulating in vitro some important aspects of native human tumors.

2. The tissue context

The roles of microenvironment in tumor development have been extensively studied in recent years. It has been observed that the surrounding osteoblasts, osteoclasts, fibroblasts and human mesenchymal stem cells (hMSC) all play essential roles in primary tumor growth and metastasis [12, 13]. Here, we briefly discuss the importance of the tissue context for tumor phenotype, and the need for an appropriate tumor microenvironment as a component of a tumor model.
Clearly, a solid tumor is far more than a collection of transformed proliferating cells forming aberrant tissue mass. Instead, they can be considered as abnormal organs composed of multiple cell types, ECM and supporting blood vessels. This particular niche, the tumor microenvironment, is a complex milieu comprising cancer cells, non-cancer cells such as inflammatory/immune cells, vascular cells, cells of mesenchymal origin (e.g., fibroblasts, mesenchymal cells), secreted factors (growth factors, cytokines, proteases), exosomes, oxygen gradients, physical signals and ECM with its laminins, fibronectin, collagen, entactin/nidogen and proteoglycans. The ECM provides structural support to the resident cells and the surrounding tissues, and acts as a reservoir of growth factors and cytokines.

Reciprocal communication between cancer cells and cells from the microenvironment is evident during tumor development. Microenvironment supports tumor initiation, proliferation, and distant colony formation and can affect how a tumor grows and spreads. At the same time, tumor cells influence the surrounding cells to show distinct abnormalities characteristic of the tumor. Importantly, tumor microenvironment has received growing attention due its role in chemoresistance, relapse and metastasis.

Therefore, it seems that creating the more biologically relevant tumor model requires recreating the tumor microenvironment component, in order to mimic a native tumor with a fidelity sufficient for studying tumor development and effects of drugs.

In the 1980s and 1990s Bissell began to model the tumor growth in 3D settings [14-17] and demonstrated that mouse primary mammary epithelium acquired organization highly similar to that observed in vivo. Using this approach, Bissell created the models of mammary gland representative both of normal tissue development and of hyperplasia, to explain how important are the environmental contributors to the initiation, progression and suppression of cancer. The key factors identified in these studies include the three dimensionality, the presence of other cells and tissue matrix, and the provision of molecular and physical signals [18,19]. These studies were the first to demonstrate that the microenvironment can both inhibit and facilitate tumor growth and metastasis [20]. Bissell’s 3D models revolutionized the field of cancer research, showing the need for complex 3D models for studies identifying cancer pathways and biomarkers [18].

### 3. Biomimetic approaches to engineering tumors

Biomimetic models of human tumors, generated using methods of tissue engineering, providing the right context for the cancer cells are now becoming a reality, largely due to the tremendous progress in understanding the native milieu of human tumors. A prerequisite for ‘engineering better tumors’ is our understanding of tumor biology, at a level necessary to recapitulate the specific features of a native tumor. By analogy with ‘organs on a chip’ engineered on microfluidic platforms to study physiology and evaluate drugs, we propose the concept of a ‘minimally functional unit’ that provides a limited but sufficient level of complexity for studying a specific aspect of the tumor.

Defining how much biological complexity is necessary for bioengineered tumor models (e.g., incorporation of stromal cells, ECM, vasculature, immune cells), and building predictable in vitro models remains a key challenge. Clearly, not the whole...
complexity of the in vivo environment can (or should) be recapitulated. A reachable goal is to develop a ‘minimally functional unit’ of a tumor, and the level of complexity of such unit will vary with the type of cancer, and the specific question being addressed. Ideally, such models should capture the essential components necessary for studying specific aspects of tumor biology, while being controllable and quantitative. Here, we highlight some advances in bioengineered models of tumors, from multicellular tumor spheroids to tissue like tumor models.

3.1 Multicellular tumor spheroids and tumor organoids

In 1966, Halpern et al. cultured cancer cells in suspension using an Erlenmeyer on a shaker – the Moscona’s technique – and showed that malignant cells have higher capacity to aggregate than normal cells [21]. For a long time cancer biologists have modeled cancer in cell aggregates, using Moscona’s technique. In 1970 Sutherland et al. studied the effect of radiotherapy and chemotherapy on aggregates of Chinese hamster V79 lung cells using spinner flasks. Aggregates generated in this way (also termed multicellular spheroids [22]) were almost perfectly spherical and contained three different zones: outer layer of proliferating cells, an intermediate zone, and a central zone of necrosis. Recent adaptations of Sutherland’s method include culturing transformed cancer cell lines under non adherent conditions such as hanging drops [23] or microfluidic devices [24]. Multicellular tumor spheroids stand out as a most frequently used in vitro model capable of recapitulating tumor heterogeneity. Therefore, multicellular spheroids are more predictive than monolayer cultures and are the most widely preclinical screening tool for anticancer drug candidates [25].

Cells of the immune system are another essential element of the tumor microenvironment, due to their pivotal role in the development, progression and treatment of human tumors. At this time, only a limited number of 3D tumor models have displayed interactions between cancer cells and immune system, which remain a major challenge for in vitro tumor models. For example, melanoma cells grown in spheroids had very limited ability to stimulate the release of immune system cytokines, as compared to monolayer cultures [26].

In 2011, a new method to culture spheroids from primary cancer cells was developed by Kondo et al. Samples of colorectal tumors were dissociated mechanically and enzymatically to isolate cancer cells and make cancer tissue originated spheroids [27] also known as ‘tumor organoids’. Tumor organoids are important for displaying the typical histological characteristics of the original tumor and for their ability for seemingly indefinite expansion in vitro [28,29].

A notable example is the model of tumor organoids developed in Ewald’s lab [30]. This group demonstrated that the ECM acts as a strong regulator of invasive tumor behavior and that tumor organoids in Collagen I hydrogel can be used to model cell invasion. Using this simple and effective assay, the group studied the invasive potential of cancer cell subpopulations from primary breast tumors and found that basal epithelial markers, including Keratin 14 (K14), are expressed in the invasive leader cells. Interestingly, they show that the bulk luminal K14 negative cells can be induced to become K14+ and lead
invasion and that knocking down the K14 gene in tumor organoids inhibits collective invasion both in vitro and in vivo.

Another notable example is organoids developed in Kuo’s lab using the colon, stomach and pancreatic cancer cells [31]. The group has developed a long term methodology incorporating an air liquid interface (ALI) for intestinal sphere like organoid cultures. The ALI configuration could achieve greater oxygenation of organoids and mimic the normal intestinal epithelial growth and multi lineage differentiation (even after a whole year in culture). Based on this system, genetically modified tumor organoids have been generated in vitro. The introduction of combinations of genes (APC-null, KRASG12D, p53 ShRNA, SMAD4 ShRNA) was shown to convert primary colon organoids to adenocarcinoma. These studies demonstrated the utility of primary cell organoids for cancer modeling and oncogene validation in gastrointestinal tissues.

3.2 Cells in porous scaffolds

In contrast to multicellular tumor spheroids, 3D tumor models formed by introducing cells into porous scaffolds are relatively new. In 2007, Fischbach et al. [32] generated the first 3D tumor model culturing oral squamous cell carcinoma in a scaffold made of synthetic poly(lactide co glycolide) (PLG). In this system, the cells attached to scaffold fibers and formed 3D structures. Importantly, the cells cultured in a 3D scaffold exhibited decreased proliferation rates and were more chemo resistant than cells grown in 2D monolayers, recapitulating at least in part the original tumor phenotype.

Since then, several models of cancer based on synthetic scaffolds (e.g., polylactide, PLA; polyglycolide, PGA; co polymers (Polylactic-co-glycolic acid], PLG and PEG, PEG hydrogels) have been generated [33]. Sophisticated synthetic scaffolds fabricated by sphere templating of poly (2 hydroxyethyl methacrylate) (pHEMA) were used to culture prostate cancer cells [34]. Dormant M12mac25 cells were re-activated to form tumors in these scaffolds but not in Matrigel. Interestingly, the scaffold recruited macrophages, which contribute to dormancy escape, suggesting that pHEMA scaffolds could be used to study tumor dormancy.

In contrast, only a few 3D tumor models have been developed using natural materials (e.g., chitosan, collagen, hyaluronic acid [HA], silk). Scaffolds made with chitosan and HA (C-HA) were used to develop glioblastoma tumors [35]. The C-HA model mimicked the native GBM niche better than either monolayers or cell spheroids and showed more cell resistance to anticancer drugs. Another model of breast cancer, generated using porous collagen I scaffolds, enabled breast cancer cells to recapitulate many of the tumor features: morphology, proliferation, overexpression of pro-angiogenic factors, and MMP transcriptions [36]. Overall, native scaffolds effectively promoted cell adhesion and growth [37].

3.3 Tissue-like tumor models

Tissue engineering brings further refinements into the 3D models of human tumors, by incorporating tissue-specific cell–cell and cell–matrix interactions, and physical signaling.
More than a decade ago, Okano’s lab developed an innovative approach to tissue engineering based on cell sheet technology [38,39]. A thermo-responsive culture plastics was used to enable reversible cell adhesion and detachment by controllable hydrophobicity of the surface. This approach allows for a non invasive harvest of cultured cells in form of an intact 3D cell sheet that preserves deposited ECM and cell–cell interactions. Recently, cell sheet technology has been applied to the generation of tumor sheets, using breast cancer as a model system [40]. As compared to subcutaneous cell injections, tumor cell sheets showed improved cell engraftment and tumor formation, presumably due to recapitulation of some aspects of the tumor microenvironment.

Incorporation of human mammary fibroblasts and breast cancer cells in a 3D co-culture model based on collagen and Matrigel allowed modeling of the progression from ductal carcinoma in situ to invasive ductal carcinoma [41]. Another step forward in increasing complexity of tissue engineered models is in the compartmentalized encapsulation of prostate cancer cells and normal prostate stromal cells in hydrogel, such that the two cell types were separated by a polymer membrane [42]. As a result, an inner core of stromal cells and an outer core of cancer cells were obtained, with normal stromal cells enhancing E-Cadherin production by cancer cells in a paracrine way.

The lack of vascularization is a fundamental limitation of most 3D models (aggregates, tumor organoids, tumor sheets, cells in scaffolds), with diffusional restrictions of nutrient and oxygen supply limiting the size of engineered tumors, and the cancer cell viability and function. Vascularization is also required for tumor metastasis. The differences between normal vasculature and vasculature in solid tumors (immature, tortuous, and hyper-permeable vessels) offers a unique target for anti-cancer therapy. A number of approaches have sought to co-culture cancer cells, endothelial cells and supporting cells in order to create vascularized tumor models. A particularly interesting tumor model was developed by tumor cells (breast and colon), human fibroblasts and endothelial cells cultured in fibrin matrix, resulting in a network of sprouting vessels, and cancer cells invasion of the surrounding matrix [43].

Tissue engineering has established a remarkable ability to design and utilize bioreactors capable of emulating many aspects of the in vivo environment. An example of the use of bioreactors in cancer research is the cultivation of myeloma explants under dynamic flow conditions, using rotating vessels [44]. These bioreactors not only preserved tumor explants over long culture times, but also maintained the architecture of skin, blood and vessels, bone and bone marrow microenvironments. This study provides a proof of principle that bioreactor cultures of tissue explants can be exploited for studying cancer biology, drug testing, and the development of patient targeted therapeutic regimes.

Another interesting example using bioreactors is the LiverChip developed by the Griffith lab [45], a commercialized perfusable bioreactor that uses a scaffold to recreate the architecture of the liver sinusoid, and human parenchymal and non parenchymal liver cells to provide the necessary cell interactions. Due its biomimetic characteristics, LiverChip seems to effectively recapitulate liver metastasis of breast cancer in vitro [46].
Microfluidic systems with human tumors perfused with culture medium are also being developed to enable more realistic studies of tumor metastasis. Recently, a human model of breast cancer metastasis into liver was established by engineering liver tumor organoids from human hepatocytes and human nonparenchymal liver cells [47]. The microfluidic platform maintained viability of tumor organoids by using micropumps coupled to oxygen sensors to generate diurnal profiles of nutrients and hormones.

4. Bioengineered bone microenvironment

In recent years, major strides have been made towards bioengineering human bone and bone tumors. Still, recreating primary and metastatic bone tumors remains a challenge because of the inherent biologic complexity of bone as a metastatic target site. We focus here on the bone microenvironment as a niche for tumor development, 3D models of bone tumors, and challenges ahead.

4.1 Bone microenvironment

Tumors in the bone can originate from the bone itself or bone marrow, or metastasize from a tumor elsewhere in the body. In all cases, the special milieu of the bone provides a niche for survival, proliferation and metastasis of cancer cells forming for example, osteosarcoma, Ewing’s sarcoma or chondrosarcoma. The metastatic process is organ specific as Stephen Paget suggested in his ‘seed and soil’ theory already in 1889 [48]. According to this theory, tumor metastasis is not due to a chance, but rather to the preferential growth of some tumor cells (the ‘seed’) in the bone microenvironment (the ‘soil’). The metastatic process is determined by highly specific interactions between disseminating tumor cells and the bone microenvironment, and metastases form only from an appropriate ‘seed’ is implanted into the suitable ‘soil’, as it occurs with breast or prostate tumor cells that go to the bone. Interestingly, bone microenvironment seems to be also a perfect ‘soil’ for dormant tumor cells to survive as single cells or micro-tumors.

In the bone, a cellular compartment consists of osteoblasts, osteoclasts, osteocytes, mesenchymal and vascular cells, and cells of the immune system. The non-cellular compartment is comprised of the organic ECM with its collagens, non collagenous glycoproteins, hyaluronan and proteoglycans, and the bone mineral. Bone has its unique properties: low pH, hypoxia, high levels of extracellular calcium and responds to a range of mechanical stimuli that are fundamental for bone homeostasis [49]. These particular characteristics make bone a suitable environment for cancer cell survival, migration, colonization and dormancy. Hypoxia and low pH in the bone control the survival and proliferation of tumor cells. Hypoxic microenvironment potentiates tumor metastasis and growth through hypoxia inducible factor (HIF)-1 that induces glycolytic enzymes, glucose transporters, and stimulates expression of the vascular endothelial growth factor, which triggers angiogenesis. Hypoxia also promotes acidosis and increased lactic acid production.

The crosstalk between tumor and bone cells, osteoblasts and osteoclasts, disrupts the equilibrium in bone remodeling through osteoclast-mediated bone resorption and osteoblast-mediated bone deposition. Bone metastases of a bone-lysing (osteolytic) phenotype reflect the inhibition of osteoblasts, of the stimulation of osteoclast function (or both) by the cancer.
cells. Metastases of a bone-forming (osteoblastic) phenotype are a result of stimulation of osteoblasts or inhibition of osteoclasts (or both) by the cancer cells. Breast cancer cells tend to be osteolytic rather than osteoblastic and on the contrary, prostate cancer cells are osteoblastic [50].

Most studies indicate that cancer cells can induce osteoclastic activation through the release of diverse soluble mediators, and most critically the parathyroid hormone-related protein that activates numerous signaling pathways driving the ‘vicious cycle’ of tumor growth and bone destruction [51]. Cancer cells can secrete factors that stimulate osteoclast-mediated bone resorption and inhibit osteoblasts, and increase the release of factors from the bone matrix that stimulate tumor growth. Hypoxia and acidosis in the bone potentiate the bone metastasis. Extracellular acidification results in increased osteoclast resorption, with contributions of the calcium released from the mineralized bone matrix to the enhanced tumor growth [52].

Interestingly, metastatic cells in bone not only remodel their microenvironment in order to survive, but also modify their phenotype trying to resemble osteoblasts. To this end, cancer cells start expressing bone matrix proteins, alkaline phosphatase, and molecules regulating the osteoblast/osteoclast cross-talk. For example, metastatic breast cancer cells express bone sialoprotein [53]. This ability to acquire a bone cell phenotype is known as osteomimicry and it is an adaptive advantage that gives tumor cells more chances to survive and proliferate into the bone tissue [54].

4.2 Engineered bone tumors

Both synthetic and natural material scaffolds have been used to recapitulate some aspects of human bone tumor biology in vitro. One notable example is the use of electrospun poly (ε-caprolactone)(PCL) as a biomimetic scaffold to show that Ewing’s sarcoma cells were able to attach to non-coated scaffolds maintaining a healthy rounded morphology and a differentiated phenotype expressing CD99 [55]. This model provided a good mimic of the morphology, growth kinetics, and protein expression profile of human tumors. As compared to monolayer culture, Ewing’s sarcoma cells cultured in PCL scaffolds were more resistant to cytotoxic drugs and showed differences in the expression pattern of the insulin-like growth factor-1 receptor, which is a target of rapamycin pathway. This study illustrated that a properly designed scaffold alone can provide important aspects of the bone microenvironment to the cultured cells and enable mechanistic studies of bone sarcomas for evaluation of drug candidates. Silk, a naturally occurring protein, has also been used to generate models of bone tumors. Metastasis of prostate cancer to bone was modeled by culturing an osteolytic prostate cancer cells line (PC3 cells) on silk scaffolds coupled with bone morphogenic protein 2, suggesting an important role of bone morphogenic protein 2 on cancer progression and prostate metastasis [56].

3D models that use synthetic or natural scaffolds provide structural and mechanical support for cancer cells, while still lacking the components of native bone environment and interactions of cancer and bone cells. Therefore, introducing bone cells into a co-culture with cancer cells is an important advance in bone tumor modeling. A notable example of such an approach is the study that combined synthetic scaffolds made of medical grade
polycaprolactone-tricalcium phosphate with cell sheet-based techniques to build a metastatic model of prostate cancer [57]. Prostate cancer cells (PC3 or LNCaP line) were cultured within scaffolds wrapped into sheets of human osteoblasts. Osteoblastic lesions (produced by LNCaP cells) and osteolytic pits (generated by PC3 cells) were observed, possibly due to an increase in MMP9 production and activation. This model provided communication between cancer and bone cells within the metastatic microenvironment of prostate cancer in bone, while maintaining a 3D conformation and mechanical properties of native bone.

A similar approach was used to model metastatic tumor cell interactions with bone by coculture of breast cancer cells within a thick osteogenic sheet generated with two lines of osteoblasts (human fetal and mouse calvaria) [58]. Phenotypic maturation of bone (osteoblast-to-osteocyte transition) was achieved using a sophisticated bioreactor that maintained osteogenic sheets cultured for prolonged periods of time (up to 10 months) without signs of hypoxia. However, this model did not mimic the mechanical environment of the bone. Nevertheless, this model recapitulated many aspects of breast cancer colonization and bone metastasis.

A bioengineered model of Ewing’s sarcoma developed in our laboratory incorporated tumor cells into the human bone like tissue that was grown in vitro using mesenchymal stem cells and native bone ECM [11]. We showed that decellularized ECM could be an ‘ideal’ scaffold for engineering functional bone tissue, as it preserves the composition, structure and biomechanics of the protein/mineral matrix of the bone [59-62]. We hypothesized that such maintenance of the structure, biomechanical properties and composition of protein/mineral matrix of the native bone will be essential for tumor survival, development and metastasis.

Micro-tissues of human bone cancer (Ewing’s sarcoma) were engineered by introducing tumor cell aggregates into their resident bone tissue, and allowing the cross-talk between the cancer cells and the human bone cells and bone matrix. The living bone niche component of Ewing’s sarcoma tumor model (TE bone) was engineered by culturing hMSC from bone marrow in 3D scaffolds made of decellularized bone in osteogenic medium for 4 weeks, using our previously established methods [63]. The tumorogenic component of the model was established using two existing Ewing’s sarcoma cell lines: RD-ES line (primary bone tumor cell line) and SK-N-MC line (primary cells originated from an Askin’s tumor – Ewing’s sarcoma generated in the chest wall – metastasizing in the supraorbital area). An additional green fluorescent protein line of Ewing’s sarcoma cells was developed from hMSCs by retroviral transductions performed using an established protocol [64]. Tumor cells were aggregated into spheroids (providing context for local cell interactions), introduced into the engineered human bone niche (providing long-range signaling), and the resulting tissue constructs were cultured for an additional 2 or 4 weeks (Figure 2).

In this 3D model, Ewing’s sarcoma cells re-express a number of genes down-regulated in monolayer cultures recapitulating in part the in vivo signature of this tumor. Importantly, after 4 weeks of culture, engineered tumors maintain the undifferentiated status and retain the CD99 marker (Figure 3A). Hypoxic and glycolytic tumor markers and necrotic areas in the inner regions of bioengineered tumors were identified already by week 2 for all three cell lines (Figure 3B), with strong increases in HIF1α mRNA relatively to both engineered bone
and cultures of tumor cells alone (Figure 3C). Finally, these models can also recapitulate the angiogenic and vasculogenic mimicry (VM). Native ES shows the presence of blood lakes and Periodic acid Shiff positive cells expressing endothelium associated genes stimulated by hypoxia (Figure 3D).

Tumors respond to oxygen and nutrient deprivation by promoting vascularization that maintains tumor growth and survival [65], with the induction of VEGF-α driven by hypoxia and mediated by HIF1-α [66]. By 2 weeks of culture, the tumor model showed high induction of VEGF relatively to both the tissue-engineered bone and the cultured tumor cells (Figure 3E). These expression levels decreased by week 4, in parallel to the decreases in HIF1-α expression, in support of the adaptive advantage of tumor cells cultured in the engineered bone. Importantly, the VEGF-α mRNA levels were not significantly increased in tumor models as compared to the engineered bone controls.

Taken together, these results suggest that within the living bone context Ewing’s sarcoma cells: i) re-express focal adhesion and cancer-related genes that are highly expressed in tumors but lost in monolayer cultures; ii) recapitulate the original hypoxic and glycolytic tumor phenotypes; and iii) acquire angiogenic capacity and VM that favor tumor initiation and adaptation.

5. Conclusion

Over the last decade, there is a growing notion on how important is the tissue environment for the initiation, progression and suppression of cancer, because cancer cells are regulated by their interactions with ECM, neighboring cells, local and systemic cues. The lack of ability to replicate the complex milieu of human cancer in vitro remains a critical barrier to more effective research and evaluation of the potential therapeutic targets. The approaches to model tumor physiology and growth are now starting to converge with those utilized in tissue engineering, and the new field of cancer engineering is experiencing tremendous advances.

Increasingly realistic in vitro models of human tumors are being developed by introducing key elements of the tumor microenvironment, such as three dimensionality, interactions between the tumor cells, healthy cells and the ECM, and physical signals. To this end, a variety of 3D culture systems: multicellular spheroids, tumor organoids, tumor sheets, and bioengineered tumor models are being ‘borrowed’ from other disciplines, along with advanced scaffolds and bioreactors. It appears that the 3D tumor models are becoming fundamental tools in cancer research that could be transformative for target identification and the development of new therapeutic options. However, cancer engineering is a relatively young field and has many challenges to overcome before the tumor models find utility in the discovery and testing of cancer drugs.

6. Expert opinion

Bioengineering methods that have transformed the stem cell research and regenerative medicine are just starting to enter the field of cancer research, by using very simple formats (multicellular spheroids, cell encapsulation in hydrogels or scaffolds) and, most recently, by
creating advanced tissue models recapitulating key features of the tumor microenvironment, including the niche components, immune cells and vasculature. Despite major progress in generating engineered tumor models, we still lack realistic in vitro models that can recapitulate the complexity of the human tumors. We list here some of the critical challenges the field is facing at this time, using bone tumors that have been highlighted in this review to illustrate specific points.

Incorporation of multiple cell types participating in bone remodeling is not always necessary for regenerative medicine applications of engineered tissues, but is important for tumor models. For example, including osteoclasts in models of bone models is a must for modeling osteolytic tumors and gaining insights on how cancer cells regulate the vicious cycle of bone metastasis.

Vascularization is another key factor. In a native bone, synergistic interactions between bone cells and vascular cells, within the 3D architecture of the mineralized bone matrix, coordinate the development and function of this complex tissue [67]. The vascular network in fact serves as a template for bone matrix deposition and is critical for both the maintenance of healthy bone and development of bone tumors. Moreover, angiogenesis is important for tumor development and metastasis due to its role in supplying cells with nutrients and oxygen, and maintaining homeostasis. Just a few of the existing tumor models incorporate blood vessels, and not a single one was developed for bone, a major target of tumor metastasis. The vascularized human bone, engineered by synchronizing vascular development and osteogenesis in cells cultured on 3D scaffolds [68], could be useful to generate vascularized bone tumors. Essentially the same methods as those established for engineering Ewing’s sarcoma tumors [11] could be used, in conjunction with perfusion bioreactors [63], to engineer vascularized Ewing’s sarcoma tumors.

Another important challenge in bone tumor modeling is to introduce components of the immune system. The mechanisms regulating the interactions among bone, immune system, and tumor cells have been deeply investigated and it is well known that T and B lymphocytes play a pivotal role in regulation of bone cell development and bone remodeling [69]. Basically, RANKL is secreted by activated T cells, to induce osteoclasts activation, whereas B cells are a major source of the RANKL inhibitor osteoprotegerin.

The roles of tumor-associated macrophages (TAMs), believed to belong to the class of M2 macrophages, in promoting tumor angiogenesis are well established [70]. Experimental evidence accumulated over the years has identified TAMs to the maintenance of a number of tumors, including breast, prostate, colon and stomach. In contrary to the expected, TAMs seem to play a role blocking and reducing bone metastasis in osteosarcomas [71]. These findings suggest that TAMs could be important anti-metastatic agents that should be included in bone tumor models.

Mechanical stimuli are fundamental for the homeostasis and growth of healthy bone, as well as for cancer progression and metastasis. Mechanical stress, fluid flow, cell matrix adhesions and cell–cell junctions coordinate tumor development as the cells are able of sensing the topography, stiffness and anisotropy of their environment. External mechanical signals are
converted into an intracellular biochemical cascade regulating gene expression, protein activity and ultimately cell function [72].

Rho GTPases are the master regulators of cell motility and cell morphology through the effects on cytoskeleton and cell adhesion, where RhoA, -B, and -C, Rac1 and -2, and Cdc42 are the best studied members of this family [73]. Over expression of RhoA, Rac1, and Cdc42 GTPases is associated with cancer initiation, progression and invasion.

Understanding the effects of mechanical stress on tumor cells and capturing mechanical loads that tumors sense when growing in the bone are major challenges in the field.

Developing robust platforms with bioengineered human tumors suitable for drug discovery is still more a goal than a reality. Miniaturization that would lead to reductions in the required amounts of cells and materials for implementation of high-throughput, multifactorial drug testing is one of the key requirements.

To maintain full control over the cell microenvironment, the bioengineered tumors need to be vascularized and perfused with culture medium. For bone tumors, the interstitial velocity should be in the range of 400 – 800 μm/s [61,74-76]. Another requirement is to determine the level of complexity needed for drug testing predictive of whole human physiology.

Even with all engineering tools we now have available (specialized scaffolds, high fidelity bioreactors, imaging modalities), we cannot possibly reproduce the whole complexity of a human tumor. Instead, we can try to define a ‘minimally functional unit’ of a particular human tumor, with components that would vary with the type of drug to be tested. A possible model of this kind would be a microtissue construct formed from cancer cells and stromal cells, around a perfused branching vascular network. The production of such microtissues should be very fast and automated.

Additional requirements include rigorous validation in drug studies, by using IC₅₀ and EC₅₀ values, and comparing these to the corresponding plasma levels measured in patients. One would expect that a reasonable benchmark for validating the bioengineered tumors in the platform would be ≤25% difference in drug responses relatively to the native tumor. Finally, the use of platforms with human tissues would be significantly advanced if online, optical readouts would be developed, for example by generating reporter lines of tumor cells.

Finally, we envision the ongoing developments in human tumor platforms as a prelude to personalized medicine, for evaluating the tumor progression for the individual patients, using their own tumor cells. We also envision that these patient-tailored platforms could be used to explore optimal drug regimens for a specific patient and state of health.

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Article highlights

- *In vitro* platforms with bioengineered human tissues are being developed to serve as physiologically relevant models for biological and medical research.
- Bioengineered human tumors provide a native-like tissue context for studies of tumor initiation and metastasis.
- The applications of bioengineered tumors in studies of tumor biology and drug discovery are at an early stage.
- Effective drug testing will require tumor models incorporating capillary bed, stromal cells, the components of immune system, and physical signaling.
Figure 1.
Differential gene expression in ESFT and monolayers of Ewing’s sarcoma cell lines. (A) Numbers of genes expressed in tumors and cell lines. (B) Focal adhesion genes and genes related to pathways in cancer that are expressed in native human tumors but not in cell lines. (C) Focal adhesion genes differentially expressed in ESFT and cell lines, by qRT-PCR. Relative endogenous expression of each gene was normalized to actin (n = 3). Data are shown for two Ewing’s sarcoma cell lines (RD-ES and SK-N-MC), three independent native tumors (ESFT) and one osteosarcoma cell line as a control of bone tumor cell line (U2OS) unrelated to ESFT.
ESFT: Ewing’s sarcoma family of tumors.
Figure 2. Bioengineered tissue model of Ewing’s sarcoma
Cancer cells, which readily lose their signatures in monolayer culture, were prepared in form of small aggregates, mimicking micro-tumors. These aggregates were introduced into human bone engineered in vitro (tissue-engineered bone TE-bone-). The human bone was formed from human mesenchymal stem cells cultured for 4 weeks in the native bone extracellular matrix. The tumor aggregates were infused into the engineered human bone and cultured in vitro for an additional 4 weeks (tissue-engineered model of Ewing’s sarcoma TE-ES-). During this time, the bone tissue context resulted in strong upregulation of cancer-related genes, and the expression of hypoxic and glycolytic tumor phenotype, and the angiogenic and vasculogenic mimicry (Figure 3).
Figure 3. Expression of Ewing’s sarcoma phenotype in the bioengineered tumor model

(A) Gross morphology of the engineered human bone (tissue-engineered bone -TE-bone-) after 4 weeks of cultivation (top panel), and the bioengineered tumor (Tissue-engineered model of Ewing’s sarcoma -TE-ES-) obtained by introducing tumor cell aggregates (after an additional 4 weeks of in vitro culture (bottom panel). (B) Expression of hypoxic and glycolytic tumor phenotype. Necrotic areas in the inner regions of the bioengineered tumor were identified by week 2 (H&E staining) in samples formed from three separate tumor cell lines: TE-RD-ES, TE-SK-N-MC and TE-EW-GFP. (C) HIF1α mRNA levels in TE-ES models. Fold change was calculated by first normalizing to actin levels in the individual samples and then to the corresponding levels in cells cultured in the monolayer. Statistical significance was determined by the two-tailed Student’s t test. *p < 0.05; **p < 0.01, ***p < 0.001; ns, not significant. (D) Representative images of PAS-stained sections from tissue-engineered bone and tissue-engineered tumor models at week 2 and 4 of culture. (E) Angiogenic and vasculogenic mimicry. VEGFα mRNA levels are shown, as fold changes relatively to the actin levels in the individual samples, that were normalized to the corresponding levels of genes in cells cultured in 2D monolayers. Data are shown as average ± SD (n = 3 5). *p < 0.05; **p < 0.01, ***p < 0.001.


ns: Not significant.