Can tissue engineering concepts advance tumor biology research?

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Advances in tissue engineering have traditionally led to the design of scaffold- or matrix-based culture systems that better reflect the biological, physical and biochemical environment of the natural extracellular matrix. Although their clinical applications in regenerative medicine tend to receive most of the attention, it is obvious that other areas of biomedical research could be well served by the powerful tools that have already been developed in tissue engineering. In this article, we review the recent literature to demonstrate how tissue engineering platforms can enhance in vitro and in vivo models of tumorigenesis and thus hold great promise to contribute to future cancer research.

Introduction

It is a sine qua non that cells in a living organism exist within a 3D environment commonly called the extracellular matrix (ECM) [1]. The ECM facilitates not only attachment between cells, the basement membrane and surrounding matrix, but also alters the transport of oxygen, hormones and nutrients, the removal of waste products, and the migration of other cell types.

However, the vast majority of in vitro studies, particularly in the cancer field, fail to mimic the 3D environment, and instead, these are performed in two dimensions, in Petri dishes, multi-well plates or glass slides that have been coated with various proteins, such as collagen, laminin and fibronectin, to simulate the ECM environment. There are several limitations associated with 2D cell culture experiments, such as lack of reproduction of physiological patterns of cell adherence, cytoskeletal organization, migration, signal transduction, morphogenesis, proliferation, differentiation and response to therapeutic stimuli (Box 1) [2–8].

Today, we can see that 3D culture using tissue engineered scaffolds and matrices (Box 2) has played a key role in the advancement of regenerative medicine over the past two decades. Although the field of tissue engineering has focused mainly to date on direct clinical applications in regenerative medicine, tissue engineering offers a potentially powerful tool box for other areas in the biomedical sciences. Among these are the establishment of more physiological in vitro and/or in vivo models that can be used to study disease pathogenesis, for example, in cancer or to develop molecular therapeutics, as well as to screen for toxic effects of drugs on human tissues [2]. These advances have been underpinned by the use of well-defined synthetic hydrogels or scaffold-based tissue engineered constructs (TECs) that generate tightly controlled micro-environments typical of the in vivo environment.

This article reviews current approaches to 3D models in tumor biology, attributes of the ECM that should be reflected in tumor cell biology models, together with the recent literature on new tissue engineering technology platforms, and demonstrates how the application of these more defined tissue-engineered microenvironments could enhance current in vitro and in vivo tumor models (Figure 1).

3D culture systems in cancer research

The vast majority of in vitro tumor biology studies are still performed using monolayer cultures even though such suboptimal study systems might result in misleading research observations and hypotheses [9,10]. However, a small yet growing number of cancer researchers, aware of the limitations of conventional 2D monolayer cell cultures, are moving towards the use of 3D cell culture systems as they begin to understand that the microenvironmental cues found in the ECM of the local cell niche, and that from other surrounding cells, are equally as important as the cell itself [8,11–16].

As early as 1985, Miller and colleagues showed that tumor cells exhibit greater drug resistance when grown as multicellular spheroids in a collagen gel compared to when grown in a monolayer [17]. Mina Bissell has pioneered the use of 3D culture systems to model the molecular mechanisms of breast cancer cell invasion by using the mammary gland as a model system [12]. Her group and others have shown that tumor cells of epithelial origin, when cultured in 3D in Matrigel (a naturally derived ECM), change shape, lose polarity and form disorganized proliferative masses or aggregates, similar to those seen in tumor progression in vivo [4,8,18]. The biological significance and clinical relevance of tumor spheroids that grow...
Box 1. Important differences between 2D and 3D cell culture systems

- Cells isolated directly from complex tissues frequently undergo a change in both metabolism and gene expression patterns when in 2D culture. The cellular structure plays a major role in determining cellular activity, through spatial and temporal ECM proteins and cell receptor interactions that naturally exist in tissues and organs. The adaptation of cells to a Petri dish requires significantly more adjustment by altering gas exchange and nutrients and waste flows and ECM interactions when compared to a 3D culture system.
- The migration of cells in the 3D environment of an organism is typically in response to a biochemical signal and/or molecular gradient. Molecular gradients, in particular, play a vital role in cell differentiation & proliferation, signal transduction and determination of stem cell fate, and countless other biological processes. It is, however, practically impossible to establish a physiological 3D gradient in conventional 2D cultures.
- Cells in a 2D environment can significantly alter the production of ECM proteins, and undergo morphological changes as a result. Cell surface receptors preferentially cluster on those parts of the cells that are exposed directly to the culture medium, rich in nutrients, growth factors, and other ligands; whereas, the receptors in cells that have been attached to the plastic surface of the Petri dish might have less opportunity for clustering. Thus, the receptors might not be presented in the correct orientation and clustering, which would affect the autocrine or paracrine signals between cells.
- In 2D culture, cells are becoming unnaturally polarized because only a diminutive element of the cell membrane can interact with the ECM and neighboring cells, while the rest of the cell is exposed to the bulk culture medium. This may lead to non-physiological, polarized integrin binding sites and mechanotransduction, which both can affect intracellular signaling and phenotypic fate.
- Differences in migration are based on the fact that a cell is confined to a plane in two dimensions, but also encounters little to no resistance to migration from the surrounding ECM. This applies to other phenomena that occur over longer time scales, such as cancer metastasis and tissue organization, in which the behaviour is regulated by mechanical interactions with the ECM.
- 3D models provide data with greater physiological relevance for mathematical models of growth, signaling, migration/invasion, and morphogenesis and for studying tumor-host interactions, such as the induction of angiogenesis and resistance to chemotherapeutic drugs.

in a 3D environment has been further well documented in the literature and summarized in several comprehensive reviews [12,19–23].

Recently, it has also been shown that 3D-cultured tumor cells display different metabolic characteristics compared to those cultured in only 2D monolayers. A predominant feature of these 3D-cultured cells is their increased glycolysis, as detected by the production of high levels of lactic acid [24]. Importantly, gene-expression profiles of cells cultured in 3D also have been demonstrated to be different from those cultured in monolayers [6]. For example, genes that encode chemokines, such as interleukin-8 (IL-8), or other factors that play a role in angiogenesis, including angiopoietin, hypoxia-inducible factor 2, and vascular endothelial growth factor (VEGF), have been found to be upregulated in tumor cells that have been cultured in three dimensions, and importantly, their gene expression pattern is comparable to that observed in tissue sections of human tumors [25]. Compared to 2D-cultured tumor cells, cells from a 3D culture also display a decreased sensitivity to apoptosis as induced by radiochemical treatment or by death receptor ligation [18,26,27]. Similarly, it has been shown that sensitivity to the cytostatic or cytotoxic effects of interferons or chemotherapeutic agents is reduced significantly in cells cultured in three dimensions [6], which suggests that other studies performed in two dimensions might not accurately reflect the chemosensitivity of tumor cells in vivo.

Thus, from a biological point of view, cancer cells cultured in three dimensions reveal a number of characteristics that sets them apart from monolayer cultures, and that more closely resemble those observed in tumors in vivo. Hence, increasingly, 3D models will be the preferred approach to assess tumor cell biology in vitro. Although some intriguing multicellular 3D models are generating some powerful new data sets [28–32], questions can still be asked as to whether the matrices used are indicative of the ECM microenvironment of that tumor type, and/or robust enough to generate reproducible data.

**Box 2. Definitions for scaffolds and matrices**

For the purpose of clear terminology, it has been proposed that hydrogels should be defined as matrices and cellular solids as scaffolds, because these terms are frequently mixed up in the literature that covers biomaterials and tissue engineering [79]. Hydrogels are made of a network of polymer chains that are water-insoluble or water-soluble but cross-linked, and often in the biomaterials field are designed as a colloidal gel in which water is the dispersion medium. Hydrogels are highly absorbent (they can contain up to 99% water) and can be fabricated from natural or synthetic polymers. In contrast, scaffolds (e.g. honeycombs or foams) are defined as cellular solids based on the definition in the engineering literature [80]. It has been argued that due to their significant larger pore size (microns to millimeter) compared to matrices (micrometer to nanometer), scaffolds serve as 2D surface with curvature for the cells [7]. However, this argument is only valid for initial cell attachment and the subsequent proliferation phase (Figure 1a) that takes place directly on the surface of the scaffolds. After the cells have covered the surface and the entire pore space is filled with cells and ECM, scaffolds represent a true 3D environment. Hence, the formerly 70% to 80% porosity of the scaffold is then filled with cells which have assembled their own natively secreted ECM. (Figure 1b).

![Figure 1. Diagrammatic interpretation of the PCL–TCP scaffold with cell seeding and attachment (a) followed by neo-tissue formation (b). Corresponding SEM photomicrographs are shown below. These SEM images clearly show that osteoblasts in combination with the mineralized matrix move into the scaffold space before exhibiting a sheet-like covering of the scaffold once fully established.](image-url)
Branes have been used extensively in cancer research. In the mid-1980s, Kleinman and Martin [33] were the first to demonstrate the value of reconstituted basement membrane from Engelbreth–Holm–Swarm mouse tumor extracts as a culture substrate, which allowed for the development of several products as a result of their pioneering work. Among these Matrigel™, which consists of mainly type IV collagen and laminin, was considered for many years to be the material of choice [34,35], based on crucial microenvironmental cues that are restored in 3D cultures of these laminin-rich extracellular matrices (lrECMs). Even though all the different lrECMs represent well the micro- and nano-scale of the native ECM, their major drawback is that they often contain residual growth factors, undefined constituents or non-quantified substances, and moreover, their batch-to-batch variations make it difficult to compare and correlate work from different groups [2,12,36,37].

Alternative systems, such as type I collagen gels, also have been used to develop 3D cancer models, because it has been shown that metastasis requires cancer cells to interact with a stromal environment that is often dominated by cross-linked networks of collagen [38–40]. Hence, to recreate an in-vivo-like environment for migrating cancer cells, researchers have embedded different types of primary cells and cell lines within 3D gels of native type I or IV collagen [38–42]. Although collagen gels can be manufactured reproducibly more easily than lrECM gels, their microstructure might nevertheless be affected by a number of factors, including their source (e.g. species or tissue location of extraction), crosslinking chemistry, temperature, pH, ionic strength and ion stoichiometry, as well as the monomer concentration. Even minor changes in these factors could significantly alter the properties of the resultant gel, thereby leading to variations of results obtained by different laboratories [40].

The multiple variables typically inherent in the use of different matrices for 3D culture systems typically makes it difficult to define not only the underlying mechanisms of changes that occur with regard to cellular phenotype and gene expression, but also in the composition of the ECM that is laid down by the cells. Native ECM is a heterogeneous collection of covalently and non-covalently interacting molecules composed primarily of proteins such as collagens, laminin, fibronectin, vitronectin and glycosaminoglycans. Chondroitin sulfate, heparan sulfate and other sulfated glycosaminoglycans are connected via covalent bonds to core proteins to give rise to proteoglycans [1]. Non-covalent interactions include electrostatic associations of ECM components with ions, hydration of the polysaccharide chains, binding of proteoglycans to hyaluronic acid (HA) and triple helix formation to generate collagen fibrils [36]. Tissue engineers offer the exciting possibility to partner with cancer researchers to design and fabricate matrices that mimic major components of the natural and tumor ECM from both a structural and functional point of view. Thus, tissue-engineered matrices and scaffolds have the ability to add clearly defined ECM elements (biochemical and biophysical) of a particular tumor microenvironment to ensure that the experimental models truly reflect major parts of their in vivo environment.

Engineered hydrogels and scaffolds for 3D cancer studies

Emerging approaches in tissue engineering have focused on the development of synthetic hydrogel-like biomaterials and scaffolds that can mimic key features of ECMs, while at the same time providing the possibility of flexibility with regard to their physical and biochemical characteristics (Box 2) [37,43,44]. Well-engineered synthetic yet biomimetic matrices are appealing because they offer lot-to-lot uniformity with more controllable and reproducible characteristics, such as matrix morphology and porosity, gel formation and cross-linking dynamics, degradation rates and mechanical properties. Such synthetic and bio-inspired hydrogels thus offer a feasible and exciting alternative to naturally derived matrices because they might help to overcome their current limitations.

The Stupp laboratory was among the first to report a self-assembling peptide system, made from natural amino acids that undergo spontaneous assembly into matrices.
with >99% water content, and a fiber diameter of 10 nm and pore sizes ranging from 5 to 200 nm [45]. Originally, the synthesis of these peptides on a larger scale was difficult and expensive, however these peptides can now be produced in milligram quantities using standard solid-phase synthesis methods and then purified to homogeneity. As a result of their inherent advantages of being able to replicate intricate biological nanostructures such as collagen fibrils, a number of self-assembling peptide systems have been developed for different biomedical applications over the years, and this work has been reviewed elsewhere [46,47].

Alternatives to natural hydrogels commonly are based on macromers or copolymers of polyethylene glycol (PEG) [48]. Upon physical or chemical cross-linking, PEG readily forms a 3D polymeric network that allows for various degrees of swelling under physiological conditions. As PEG by itself is not susceptible to degradation, functional units have been introduced that can be cleaved enzymatically or via hydrolysis as noted below. Rizzi et al. [49,50] have described a unique PEG-based hydrogel platform that allows the design of its biological and physicochemical material characteristics at the molecular level, for example, degradation by cell-derived specific ECM proteases, such as matrix metalloproteinases, or the incorporation of adhesion peptides (e.g. the RGD motif), which mimic cellular integrin receptor binding sites on ECM proteins. Using such 3D matrices, new 3D in vitro models for ovarian [51] (Figure 2) and prostate [52] cancer research can be developed that allow analysis of cancer cell function in a more defined tumor microenvironment. Several other groups have bioengineered similar matrices, as reviewed elsewhere [37,43,53]. Overall, this molecular engineering of artificial ECMs composed of PEG aims to provide a controlled cell environment to instruct cell behavior and enable complex processes of tissue formation and regeneration to be more controlled [7].

Another gel system originally developed for tissue engineering applications that consists of RGD-peptides coupled to alginate and that also allows control over gel mechanical properties has recently been used in cancer biology studies [54]. Strikingly, 3D culture within this system best reflected in vivo experiments and led to enhanced IL-8 secretion from oral squamous carcinoma cells, which was dependent on integrin binding in three but not two dimensions. In contrast, VEGF secretion was not significantly influenced by 3D culture in the presence or absence of integrin engagement, or in vivo, despite increased levels observed on 2D culture [54].

These results demonstrate the power of synthetic tissue-engineered 3D culture systems, not only to perform more biomimetic mechanistic studies, but also to identify

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Figure 2. Ovarian cancer cells grown in 3D hydrogels. (a) Schematic illustration and examples of cancer cells grown in 2D monolayer cultures, embedded in synthetic 3D hydrogels and in co-cultures using fibroblasts, indicate the spheroid formation observed on 3D culture and close proximity of cancer cells and fibroblasts in 3D co-culture, thus mimicking the in vivo microenvironment (scale bars 100 μm); actin filaments are stained with phalloidin rhodamine (red). (b) This panel shows cell colony formation of OV-MZ-6 ovarian cancer cells in PEG hydrogels constituted with and without the RGD peptide integrin motif, and that increased spheroid formation occurs with RGD hydrogel incorporation, which indicates a requirement for integrins in spheroid formation and growth. Cells were imaged on days 1 and 14 using phase contrast (left hand panels) and laser scanning confocal microscopy (right hand panels) (scale bars 100 μm); actin filaments were stained with phalloidin rhodamine (red) and nuclei with DAPI (blue). (c) Enhanced proliferation of OV-MZ-6 cells upon RGD functionalization of synthetic PEG hydrogels is confirmed by cell DNA content measured using quantitative CyQuant assays. (d) Cellular morphology of the spheroids was examined using histological sectioning and hematoxylin and eosin (H/E) (50 μm) and toluidine blue (20 μm) staining, SEM (20 μm) and TEM (2 μm, zoom 0.2 μm) analysis confirmed the structural integrity of these cellular spheroids within the tissue-engineered PEG hydrogel matrix. AC, apoptotic cell; ER, endoplasmic reticulum; N, nucleus; TJ, tight junction.
promising therapeutic targets. For example, early events in tumor growth, in particular those occurring prior to vascularization, appear to be more closely reproducible in 3D culture systems \[54\]. 3D cultures of tumor cells typically develop hollow cores that resemble the necrotic areas of in vivo cancers; areas that are usually at some distance from nutrient and oxygen supplies \[55\]. Furthermore, the proliferation rate of 3D-cultured tumor cells is often slower than observed in 2D culture, and more physiologically relevant than that of monolayer cultures. Moreover, and one of the most significant advantages of 3D cultures, is that they allow the exploration of the interactions of different cell types (Figure 3). For instance, infiltration of tumor spheroids by endothelial cells has been shown to depend, not only on the production of pro-angiogenic factors by tumor cells, but also on the expression of cadherins by endothelial cells \[56,57\].

As an alternative approach to hydrogels, scaffolds can be used for 3D cell culture systems. Mooney’s group \[55\] was one of the first to report in vitro and in vivo experiments that used scaffolds originally developed for tissue engineering purposes in combination with human carcinoma cells. The foam-like scaffold employed was made of poly(lactide-co-glycolide acid) (PLGA). Similar to their alginate–RGD model above, the angiogenic characteristics of the oral squamous carcinoma cells (increased IL-8 and decreased VEGF) seeded in this scaffold were similar in the 3D in vitro model and in vivo tumor xenograft experiments, but different from that observed in routine 2D cell culture.

The Hutmacher/Clements laboratories have employed a number of scaffold and matrix technologies to generate 3D in vitro models (Figure 3) for breast and prostate cancer bone metastasis research \[52\]. For example, one approach uses a medical grade polycaprolactone-tricalcium phosphate (mPCL–TCP) scaffold wrapped with a sheet of human primary osteoblasts similar to that reported previously for bone regenerative studies \[58\]. The cells attach to the scaffold, proliferate, and eventually fill the pores of the scaffold, thus generating a new osteoblast-derived bone matrix (Figure 3) to which cancer cells can be added to assess tumor–bone interactions.

Although advances in biomaterials research are driving the development of novel biomimetic hydrogel and scaffold platforms, moving cell cultures into the third dimension is not without challenges. The routine methods for imaging and analyzing cell function and protein distribution are much more complex within the 3D microenvironment, although achievable as demonstrated elegantly by the Sloane group \[59\]. In addition, in hydrogels, there is more limited access to the cells for immunoassays or DNA/RNA extraction, and secreted proteins can sometimes be difficult to extract from these constructs. Imaging methods, such as optical, fluorescence and confocal laser scanning microscopy, become complex as light scattering, refraction and attenuation mean that acquisition of high quality images is challenging and requires substantial optimization work depending on the matrix used. Similarly, specimen preparation for scanning electron (SEM) and
transmission electron microscopy (TEM) (Figures 2 and 3) are challenging technically because of the fixation and dehydration steps. Most important, methods to release cells, proteins and other biological molecules from 3D constructs need to be considered with these platforms and validated to perform the same sophisticated biological assays that are routine in two dimensions.

**Bioreactors**

For some time already, bioreactors have been considered as a feasible means to produce viable tissue-engineered construct (TEC) by culturing cells and bioactive factors within a scaffold or hydrogel in a hydrodynamic microenvironment [60–62]. Bioreactors provide a fluidic environment for tissue-engineered constructs to promote their viability, maturation, easy bio-monitoring and testing, storage and transportation. Different types of bioreactors have become available that vary greatly in their size, complexity, and functional capabilities, and this work has been reviewed in detail in other articles in this journal [63,64].

Perfusion bioreactors, which force the medium flow through the internal porous network of the tissue-engineered constructs, thus mitigating internal diffusional limitations, is one approach [65,66]. A bioreactor originally developed for bone engineering applications permits growth of 3D multiple cell-layered osteogenic tissue from murine osteoblasts over long, continuous-culture intervals. This bioreactor has been used to study the interaction between cancer cells and the in vitro engineered bone-like tissue in real time. Metastatic human breast cancer cells, MDA-MB-231 (GFP), introduced into the model grew and colonized osteoblastic tissue in a manner that reflected some of the characteristics of pathological tissue from breast cancer bone metastasis observed clinically [67]. Similar bioreactors have been developed for melanoma and prostate cancer models [68,69].

Thus, bioreactors can provide additional technical means to perform controlled studies aimed at understanding specific biological effects related to tumor biology and metastases. Hence, bioreactors originally developed for tissue engineering applications have the potential to further enhance 3D culture technology platforms in cancer research and could open up a new research field.

**Tissue engineered in vivo cancer xenograft models**

As microenvironmental conditions are known to regulate tumorigenesis, in vivo model systems that use tissue-engineered biomimetic platforms that replicate specific attributes of the tumor microenvironment might aid in understanding their influence on cancer growth and metastatic spread (Box 3). It is known that factors, such as alterations in interactions between cells and ECM, angiogenesis, development of hypoxia, or signaling of cells that reside within spatially distinct and tissue-specific niches, enhance tumor aggressiveness, but these underlying conditions are often only partially reflected by currently applied in vivo model systems [70]. This further emphasizes the need to develop a new generation of improved cancer models that better reflect clinical phenotypes, but which are still amenable to detailed cellular and molecular biology studies [71–73]. However, it is necessary to keep in mind that engineering a permissive construct that promotes tissue formation of choice and/or remodeling in vivo might be preferable over the complete in vitro engineering of a complex tissue. Similarly, it might be unnecessary to exactly replicate the complexity of the native ECM. A more pragmatic approach would be to bring together a TEC that is as simple as possible and then use the animal body as a bioreactor. It might be important, however, to first assemble the correct set of components inside a TEC, such as an appropriate scaffold, exogenous cells and/or cytokines/growth factors [74].

Such an approach employing PLGA scaffolds that had been originally developed for tissue engineering purposes was recently used as noted above to pre-condition the tumor cells to an in vivo environment before seeding in the host mouse [55]. An important observation is that, because the angiogenic characteristics of the tumor cells are dramatically altered in the 3D culture microenvironment and resemble more closely those of in vivo tumors, preculture in three dimensions could induce greater malignant potential. Additionally, tumors generated with the 3D-cultured cells are more resistant to cytotoxic drugs in vivo, which better replicates the clinical situation and thus provides a better model for drug testing [55].

Another group has developed an injectable, in situ cross-linkable, semi-synthetic extracellular matrix (sECM), derived from HA, to deliver and grow cancer cells in vivo [75]. The HA-derived sECMs were seeded with either breast, colon, or ovarian cancer cells prior to gelation, and then injected subcutaneously into mammary fat pads, sub-serosally into colons, and intracapsularly into ovaries, respectively. The authors concluded that delivery of cancer cells in sECM hydrogels showed advantages such as increased incidence of cancer formation and reduced variability in tumor size, enhanced growth of organ-specific cancers with good tumor-tissue integration combined with reduced cancer seeding on adjacent tissues.

**Box 3. Paradigm shift from 2D to 3D techniques, the way forward for the 21st century**

We do not argue against the fact that 3D cultures fail to reproduce entirely the enormous complexity of cancer cell biology in vivo. Thus, it is unlikely that they will ever be able to replace totally experimental in vivo models in the analysis of the molecular mechanisms that underlie cancer and tumorigenesis. However, we emphasize that in vitro 3D tissue models, specifically the ones derived from tissue engineering research, are very useful experimental tools for cancer research. Although some 3D models aim at the reconstitution of living tissues, they are not intact animals and for that reason do not reproduce the entire normal in vivo microenvironmental anatomy and physiology. For example, transport limitations of oxygen and other nutrients, and pH can become important in these avascular models. As such, 3D systems must be considered models, and conclusions from the in vitro data gained from them may require further testing in vivo. Nevertheless, they provide potentially powerful tools for new applications represented by the examples described in this article. As described here, a paradigm shift from 2D to 3D techniques should be the way to go in the 21st century. A number of laboratories and companies have begun to develop and apply technology platforms to energize this paradigm shift and a variety of natural, synthetic, or semi-synthetic materials are now available.
The ability of malignant cells to migrate from a primary site adherent to the bone vasculature, extravasate, and to then establish a secondary tumor colony is termed osteotropism [76]. This mode of cancer metastasis is restricted primarily to breast and prostate cancers although others, such as lung, thyroid, kidney, and multiple myeloma may also metastasize to bone. However, only a limited number of in vivo models exist, which allow one to assess bone development rapidly or to study tumor progression in a more human-like environment in an animal model. These have been generated primarily by the tissue engineering community. In a recent study [77], a hydroxyapatite scaffold preloaded with the bone-inducing factor, bone morphogenetic protein-2 (BMP-2), was vascularized surgically with a ligated murine femoral artery. Over time the presence of bone-like tissue in the BMP-2 treated tissue engineered construct was observed as well as a significantly enhanced growth rate of PyMT-cells (an osteolytic inducing mammary tumor cell line) implanted 6 weeks after the initial scaffold [77], thus indicating the potential of this model to be further validated for bone metastasis research.

Silk scaffolds that had been coupled with BMP-2 and/or seeded with bone marrow stromal cells also have been used to study bone metastasis [76]. However, these constructs were implanted subcutaneously on mouse flanks with the aim of pursuing an orthotopic breast cancer metastasis model with human breast cancer SUM1315 cells implanted into the mouse mammary fat pads, and homing to the bone scaffold implants followed. The tumor burden in the subcutaneously implanted scaffolds to which the SUM1315 cells had homed was greatest in the scaffolds treated with BMP-2 or bone marrow stromal cells and cultured for just 1 day in vitro, which suggests that a less differentiated or a bone stem cell environment is required to support metastatic growth [76]. These experiments are some of the first successful examples of in vivo engineered tissues as a model system of human breast cancer metastasis.

With respect to prostate cancer bone metastasis studies, recent models also have focused on providing a human bone microenvironment within a mouse model. The original studies used human bone chips implanted subcutaneously in NOD/SCID mice and seeded prostate cancer cells after 4 weeks to allow for vascularization of the bone chips [78]. Although intuitively an ideal approach, it is often difficult to obtain clinical samples (taken from patients which undergo hip or knee prostheses replacement surgery) that are still highly viable and will vascularise in vivo, hence tissue engineered bone might be an attractive alternative. It was previously shown that a scaffold/cell sheet construct can be used to engineer bone in a nude rat model in a highly reproducible manner [58]. Both cancellous and cortical bone can be engineered ectopically, depending on the scaffold design and cell type (osteoblast versus cancellous bone marrow stromal cells), culture time and time of implantation. Ongoing research in one of our groups (Figure 3) is aimed at using this construct (a medical grade PCL-TCP scaffold wrapped with a human osteoblast sheet) and comparing engineered human bone to the standard bone chip model in NOD/SCID mice. These studies indicate that LNCap prostate cancer cells can grow successfully within the scaffold in vitro and in vivo (Figure 3), thus anticipating that such a system will provide a potential new human to human (human cancer cells in human bone) model for studies of bone metastasis.

Conclusions

The studies discussed in this article illustrate the potential of 3D culture concepts, which were developed originally for tissue engineering applications, as model systems to enhance cancer research. In particular, these models provide a more controlled environment to add specific cell types, ECM proteins, growth factors and other inductive factors to hydrogels and/or scaffolds to better mimic tissue and tumor-specific niches that favour primary tumor and metastatic growth. These models will likely be of significant use in elucidating our understanding of the fundamental biological processes that direct the pathological abnormalities observed in cancer. They might also provide a unique platform to discover previously unappreciated biomechanical influences, an area well researched by tissue engineers, on cancer formation and homeostasis. Nevertheless, current 3D culture models do have inherent limitations in entirely mimicking the tissue behaviour in vivo. Further improvements of 3D culture systems, particularly the development of innovative heterotypic co-culture strategies and scaffolds and/or matrices that are easily manipulated, will be invaluable in accurately modeling cancer progression and in testing novel therapeutic strategies within a biologically relevant context.

Acknowledgements

DWH and JAC thank the Prostate Cancer Foundation Australia for support of their research. DL, DWH and JAC thank the National Health and Medical Research Council (NHMRC) of Australia for funding support. DK thanks the NIH (Tissue Engineering Resource Center) and the Susan Komen Foundation for support of their research. DM thanks the NIH (RO1-DE019917) and the DoD (BC084682 Idea Award). DWH and JAC would like to acknowledge the contribution of their PhD students Shirly Sieh, Parisa Hesami, Verena Reichert and vet-surgeon Siamak Safdazeh.

References

Review


