3D tumour models: novel in vitro approaches to cancer studies

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Abstract 3D in vitro models have been used in cancer research as a compromise between 2-dimensional cultures of isolated cancer cells and the manufactured complexity of xenografts of human cancers in immunocompromised animal hosts. 3D models can be tailored to be biomimetic and accurately recapitulate the native in vivo scenario in which they are found. These 3D in vitro models provide an important alternative to both complex in vivo whole organism approaches, and 2D culture with its spatial limitations. Approaches to create more biomimetic 3D models of cancer include, but are not limited to, (i) providing the appropriate matrix components in a 3D configuration found in vivo, (ii) co-culturing cancer cells, endothelial cells and other associated cells in a spatially relevant manner, (iii) monitoring and controlling hypoxia- to mimic levels found in native tumours and (iv) monitoring the release of angiogenic factors by cancer cells in response to hypoxia. This article aims to overview current 3D in vitro models of cancer and review strategies

employed by researchers to tackle these aspects with special reference to recent promising developments, as well as the current limitations of 2D cultures and in vivo models. 3D in vitro models provide an important alternative to both complex in vivo whole organism approaches, and 2D culture with its spatial limitations. Here we review current strategies in the field of modelling cancer, with special reference to advances in complex 3D in vitro models.

Keywords Biomimetic · Tumour stroma · 3D cancer models · In vitro tumour models

List of abbreviations

2D	Two-dimensional
3D	Three-dimensional
bFGF	Basic fibroblast growth factor
BME	Basement membrane extract
BSA	Bovine serum albumin
DOX	Doxorubicin
EC	Endothelial cell
ECM	Extracellular matrix
EGF	Epidermal growth factor
EHS	Engelbreth-Holm-Swarm
EOC	Human epithelial ovarian cancer
HA	Hyaluronan / hyaluronic acid
IL-8	Interleukin-8
lrECM	Laminin-rich extracellular matrix
MCS	Mesenchymal stem cells
MCTS	Multicellular tumour spheroid
MMP	Metalloproteinase
NOD	Non-obese diabetic
PBS	Phosphate buffered saline
PC	Plastic compression
PGA	Polyglycolide
PEG	Polyethylene glycol
PLA	Poly lactide

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PLG/PLGA	Poly(lactide-co-glycolide)
PVA	Poly(vinyl alcohol)
RGD	Arginine-glycine-aspartic acid
SCID	Severely compromised immunodeficient
VEGF	Vascular endothelial growth factor

Introduction

Cancer is one of the major causes of death worldwide, accounting for around 7.9 million deaths in 2007 (World Health Organization 2011). A solid cancer, such as those of the breast, colon, stomach, lung and liver, is a tumour mass which is hypoxic at the centre and denser than the surrounding tissue. On the cellular level, cancer is characterised by the escape of cancer cells from normal growth control and the acquisition of an invasive potential. This is accompanied by the creation of a supporting stroma, including an increased vasculature. For cancers to grow and invade, favourable chemical interactions take place between cancer cells and tumour stroma. Together, these create a clinically detectable invasive tumour mass characteristic of solid tumours.

The majority of cancers (carcinomas) originate from epithelial cancer cells which form cancer foci. These interact dynamically with the surrounding stroma, which contains

non-epithelial cells, mainly fibroblasts and endothelial cells (ECs, lining blood vessels) and the extracellular matrix (ECM) which is rich in glycosylated proteins (Fig. 1). Epithelial-stromal interactions are crucial for growth and progression. For example, the cancer mass cannot grow beyond ~200 µm in diameter without a blood supply (Calmels et al. 1995). Non-availability of oxygen results in hypoxia-inducible factor (HIF1α) upregulation within cancer cells. This in turns promotes transcription of proteins of the angiogenic factor cascade, including vascular endothelial growth factor (VEGF), which are regulated in a temporally distinct manner. The latter attracts ECs towards the cancer cells to form new vessels, to supply the proliferating cancer cells with adequate nutrients and O₂. The cycle of growth and vascularisation is propagated by the hypoxic centre of the cancer mass. Tumour stroma is also populated by inflammatory cells (macrophages, neutrophils and mast cells). These contribute to tumour aggressiveness via stimulation of angiogenesis, since they synthesize pro-angiogenic factors (e.g., VEGF and basic fibroblast growth factor bFGF) on demand at specific locations (Pollard 2004). Resultant expansion requires remodelling of the surrounding ECM, regulated by metalloproteinase (MMP) enzymes, usually produced by fibroblasts and macrophages. MMP-2 and MMP-9 are often upregulated in a variety of cancers and

Fig. 1 Tumour mass with stroma: showing the major supporting non-cancer stromal cells and pivotal protein families. Individual cancer cells not shown

degrade collagen IV in the basement membrane, which forms a boundary for the tumour, to allow migration (Tian et al. 2005). Cancer cell invasion is often driven by ECM proteins involved in cell-cell and cell-matrix interactions, such as integrins. They play a key role in metastasis by sending and receiving molecular signals, via cascades like the mitogen-activated protein kinase pathway or the focal adhesion kinase signalling pathway, which control the invasive and metastatic progression of malignant cells (Hemler et al. 1996; Dedhar and Hannigan 1996).

Pre-clinical research to delineate molecular mechanisms that drive cancer growth and progression or to determine efficacy of experimental therapeutics is usually carried out in two-dimensional (2D) cancer cell cultures or in in vivo models. Both carry advantages and disadvantages.

Molecular and genetic mechanisms underlying cancer have been widely studied in 2D models using cultured cancer cells usually growing as monolayer. For example, a large number of studies have delineated different specific steps on how alterations of oncogenes and tumour suppressor genes contribute to oncogenesis. A case in point is the deregulation of genes belonging to the *myc* family, especially *c-myc* whose gene product is a transcription factor. Mutations result in altered regulation of target genes and drive genomic instability and increased angiogenesis. Therefore *c-myc* mutations play a pivotal role in tumour growth and progression of a large number of different cancer types, like melanoma and prostate carcinoma (Ponzielli et al. 2005; Albiñ et al. 2010). Co-cultures of different cell types are also routinely used where for example cancer cells and fibroblasts or ECs are grown in a “paracrine configuration”, with cells kept in distinct locations using well inserts in a well-plate, to mimic the signalling interplay between cell types. A co-culture system investigated gene expression of urokinase plasminogen activator (uPA) (a protease which drives ECM refashioning and is associated with rapid disease progression) in stromal fibroblasts while under the influence of ovarian cancer cells (Noskova et al. 2009). This study identified bFGF and epidermal growth factor (EGF) as cancer-produced paracrine factors which stimulate uPA expression by stromal fibroblasts. Other tumourigenic associations described in co-culture systems include rapid self-organisation of both mesenchymal stem cells (MSC) into channel-like structure and neuroblastoma cancer cells into island foci, when the two cell types were co-seeded; moreover the cancer cells exhibited prolonged viability in the presence of stem cells compared to viability of cancer cells alone, under oxidative stress (Rizvanov et al. 2010). Also enhanced invasion of breast cancer cells (MDA MB 231) was observed when co-culturing them with MSC derived from adipose tissue (Pinilla et al. 2009). However, overall, the reductionist 2D approach does not mimic the native in situ environment of cancer or normal tissues, or recapitulate 3D cell morphology and may distort cell-integrin interactions (Cukierman et al. 2001).

In vivo models have the advantage of providing the native 3D microenvironment in which tumours reside. The most routinely used in vivo model is the human tumour xenograft which consists of inoculating human cancer cells or small fragments from cancer specimens either subcutaneously or in other organs/tissues of the laboratory mouse (*Mus musculus*) and allowing the tumour to grow (usually 1–8 weeks). A great advantage of this method is the capability to transplant both cancer tissue and surrounding stroma to mimic the complexity of the human tumour environment. Mice used for xenografts are immunocompromised so they do not reject human cells and include: athymic nude mice, severely compromised immunodeficient (SCID) mice or non-obese diabetic (NOD)/SCID humanized mice (Richmond and Su 2008). Tumour growth is often challenging in athymic nude mice, with no tumour progress observed even after 6 months (Elkas et al. 2002). In SCID mice tumour development occurs in most of the cases and NOD/SCID humanized mice are able to mimic immune response similar to that of native tumours. Nevertheless, a major challenge in xenograft models is how to observe tumour growth or regression over a course of the treatment, for example at weekly intervals. Assessment of treatment efficacy can dependently be performed at the end of the trial, when the animal is sacrificed (Nilsson et al. 2002). However with advances in in vivo animal imaging, monitoring of response to treatment is now possible, albeit expensive and often difficult to source (Loudos et al. 2010). Xenograft models have been useful in therapeutic testing, e.g., the classical experiments reporting the action of the angiogenic inhibitor endostatin (Boehm et al. 1997). The agent was administered to three groups of mice growing different tumours (Lewis lung carcinoma, fibrosarcoma and melanoma). All tumours regressed significantly. Tumours were allowed to re-grow up to six times and were treated each time successfully with endostatin, without developing resistance against this drug. However, phase I clinical trials revealed that endostatin works differently in humans. Although no toxicity was reported, there was no significant biological activity, with only three patients out of 15 demonstrating therapeutic benefits. In one patient tumour showed slow regression (17% over a year) and tumours in two patients remained stable (Eder et al. 2002). This again demonstrates how in vivo models may provide insufficient relevant information for translation to the clinic.

3D culture models range from simple cancer cell spheroids to models comprising multiple cell lines. They are used in an effort to mimic more closely the tumour microenvironment, and provide a compromise between the reductionist approach which isolates cancer cells as a 2D monolayer and the manufactured complexity of growing human tumours in xenogeneic hosts. A widely used strategy is to propagate cells in tissue culture and then implant them in a 3D matrix scaffold as single cells or aggregates. As

there is no blood supply, 3D models allow only for a simple diffusion of nutrients and oxygen to the cell aggregates. This gives an upper limit to the size of cell aggregate that can be useful. However, nutrient restriction may better reflect the tumour microenvironment than a fully oxygenated and nourished cell layer. Below we give an overview of different types of 3D tumour models with specific emphasis on recent promising developments.

Multicellular tumour spheroids as a primary 3D tumour model

Multicellular tumour spheroids (MCTS) consist of cancer cells from established cell lines or disaggregated human tumour fragments. They can be studied in suspension in bioreactors or in 3D matrices and closely resemble cell-cell and cell-matrix interactions in vivo (Sutherland 1988). MCTS consist of actively proliferating cells on the outside of the spheroid with quiescent cells in the inner, nutrient-deprived zone (Freyer and Sutherland 1980). Therefore, the spheroid size is limited to 400–600 μm as with increasing size the cells inside the spheroid become necrotic (Kunz-Schughart et al. 1998; Mueller-Klieser 1997). This is likely due to the limited O_2 and nutrient availability, mimicking the natural scenario in a tumour in situ. Most importantly, the 3D structure of MCTS creates a penetration path which needs to be overcome by any agent to be tested, therefore MCTS are often used for testing of chemotherapeutics.

Multicellular tumour spheroids are formed naturally from cancer cell lines when they are rotated slowly (50 rpm) at 37°C in a humidified environment. Ong and colleagues (2010) reported a novel method for the creation of MCTS. Spheroids from human hepatoma cells were linker-engineered using a transient inter-cellular linker solution which promoted cell-cell interaction and aggregation. The “linker” was manufactured using polyethylenimine (PEI) which contained multiple arms on a positively charged backbone. Chemical groups on the “arms” reacted with cell surfaces to bring cells together within half an hour, with the linker having a half-life of about 2 days (Ong et al. 2010). To assess that spheroids, as opposed to cell aggregates, were truly formed, four key properties were investigated in the linker-engineering constructs: (1) cell-cell interactions, proved by the presence of e-cadherin, one of the major integrins responsible for cell-cell interactions; (2) development of extracellular matrix (ECM), confirmed by the presence of fibronectin, laminin and collagen, key components of the ECM; (3) gradients of nutrient concentration, demonstrated by the detection of hypoxic regions and (4) cell proliferation, which showed that linker-engineered MCTS developed in less than half the time needed for rotator-formed MCTS (7 days versus 15 days).

The major applications of MCTS models are in testing chemotherapeutic agents, in particular testing novel drug delivery systems, and mapping response to treatment. For example, MCTS from MCF7 human breast cancer cells were used to study uptake of PET tracers: 2- ^{18}F fluoro-2-deoxyglucose (FDG) and ^{18}F 3'-deoxy-3'-fluorothymidine (FLT) as a potential indicator of treatment response in breast cancer (Monazzam et al. 2006; Monazzam et al. 2009). Novel drug delivery systems have been designed to prevent non-selective drug accumulation and low half life of a chemotherapeutic. One of the more effective methods has been encapsulation of the cancer chemotherapeutic Doxorubicin in a polymeric micelle which has shown greater penetration ability through the human cervical carcinoma MCTS than free Doxorubicin (Kim et al. 2010). Another approach involved using novel pH-responsive endosomolytic polymers, synthesized by modifying poly(L-lysine isophthalamide) with amino acid L-phenylalanine (Chen et al. 2009), together with calcein as a model drug. As tumour pH decreased from the exterior to the interior of MCTS pH-responsive polymers degrade while diffusing through them and stimulated calcein fluorescence by promoting its release from the intracellular endosomes (Ho et al. 2011).

Engineering the 3D microenvironment: scaffolds for 3D cancer studies

Tumour is characterized by the disordered structure of ECM and unregulated cell growth. Cells present within the tumour interact not only in autocrine or paracrine fashion but also with ECM components. By culturing cancer cells with specific ECM constituents, it is possible to replicate some of the in vivo cell-cell and cell-ECM interactions (Lee et al. 2007). Interestingly, direct contact with specific ECM components affects epithelial cancer cell behaviour. A comprehensive study by Kenny et al. 2007 used laminin-rich extracellular matrix (lrECM) substrata to study morphology and gene expression of 25 epithelial cancer cell lines derived from mastectomy and breast tumours. After 4 days incubation, resultant cell morphology was divided into four groups: round, mass, grape-like and stellate. Cells in each group were characterized by different properties: (1) the stellate group exhibited increased invasiveness and this was confirmed at the molecular level by the absence of E-cadherin expression; (2) the grape-like and the mass groups demonstrated increased aggressiveness and metastatic potential which was supported by high levels of the Erb2 receptor; (3) the round group showed no invasiveness, and at the molecular level this was accompanied by low levels of Erb2 receptor and high levels of E-cadherin (Kenny et al. 2007). The ECM substrates provided a more responsive

microenvironment than tissue culture grade plastic. However just the presence of these components, without true recapitulation of the 3D environment does not encourage the native scenario where cells migrate through matrix to form clusters, i.e. the precursors of a tumour.

Cell excreted ECM as a 3D matrix

Certain cell types (e.g., epithelial, endothelial) produce and deposit components of basement membrane proteins. Basement membrane is a thin layer of specific ECM components that supports and lines the epithelium and endothelium. The basement membrane is visible under microscopy as a separate structure from the rest of the ECM. It contains proteins which engage in cell-matrix interactions to regulate migration, attachment, repair and spread of cells, the latter being a pivotal step in tumorigenesis (Fan et al. 1987). Hence, the basement membrane proteins, which include laminin, collagen IV and entactin, are critical for regulating cell behaviour.

During the culture of Engelbreth-Holm-Swarm (EHS) tumour cells, the deposited basement membrane proteins are collected and form the basis of a mixture, which is commercially available as Matrigel (Kleinman and Martin 2005). This mixture or extract forms a 3D gel through aggregation of proteins when heated to 37°C, and can be used for cell culture. As an example of a common application, both cancerous and non-cancerous human prostate epithelial cell lines were grown in Matrigel. The non-cancerous cells were characterized by formation of acini (cell clusters), which resemble in vivo structures of prostate epithelial cells. Cancer prostate cells showed lack of organization and increased invasiveness, which is associated with cancer aggressiveness (Webber et al. 1997). Matrigel constituent proteins promote cell-matrix interactions, affecting cell proliferation and differentiation. For example, rapid proliferation of human HT1080 fibrosarcoma cells was demonstrated in Matrigel (Kramer et al. 1986). This high proliferation rate demonstrates the importance of the basement membrane proteins. However, the results also highlight the need to accurately mimic the correct ECM type, ratio of constituents and density if we are ever to truly mimic the tumour environment in vitro to study natural cell behaviour. Matrigel does not contain high proportions of collagen type I or hyaluronan, which are found throughout the ECM of tumours in vivo at relatively high proportions. By eliminating these proteins from the milieu, specific native cell behaviour and responses may not be recapitulated, especially considering the importance of the structural role of proteins like collagen I in maintaining tissue architecture. Matrigel has also been used for studying interactions between fibroblasts and cancer cells. Tumour-associated fibroblasts contribute to tumour invasiveness by producing

MMPs which degrade the basement membrane. Breast preneoplastic epithelial cells when co-cultured with tumour-associated fibroblasts showed induced growth and ductal-alveolar morphogenesis. Normal fibroblasts were unable, or weakly able, to influence epithelial cell behaviour or morphology (Shekhar et al. 2001).

An alternative approach to mimic the native in vivo tumour microenvironment, is to use fibroblast-derived 3D matrices. Fibroblasts harvested from stroma at different stages of tumour progression of an in vivo model do not retain their different characteristics in a 2D model, like growth and ECM secretion, such as desmoplastic markers, but exhibited them in a 3D model. Furthermore, the secreted 3D matrices determined the morphology of newly seeded fibroblasts, for example the “advanced tumour” 3D matrix induced desmoplastic characteristics (Amatangelo et al. 2005). Adenocarcinoma cell lines showed distinct aggregation, growth, proliferation and morphology profiles on fibroblast-derived 3D matrices, which did not necessarily correlated to 2D behaviour (Serebriiskii et al. 2008) and were grouped into different classes, depending on the degree of proliferative versus morphological response. However, a disadvantage is that fibroblast-derived 3D matrices did not support cell growth efficiently, with some cells showing slower growth rate, like the breast cancer cell line MCF-10A (Castello-Cros et al. 2009). This type of 3D matrix is an important model of cell-matrix interactions, however, like Matrigel, it does not fully represent the composition and structure of the tumour microenvironment.

3D tissue-engineered approaches

A 3D scaffold is a temporary structure that supports cells in a given environment, which may eventually be incorporated into the tissue. Scaffolds have been widely used in tissue engineering, for the purpose of wound healing or bone regeneration (Hutmacher 2000; Agrawal and Ray 2001). They have been adapted as matrices for cell culture and for the investigation of proliferation, growth and migration of cancer cells (Table 1). Scaffold properties, including composition, configuration and porosity dictate the extent to which cells can migrate, proliferate and aggregate. As a 3D structure, a scaffold has the potential of recapitulating the native geometry, unlike 2D cell monolayer (Fig. 2). This subsection has been divided into two main categories, depending on the main component of the scaffold: natural and synthetic scaffolds.

Natural scaffolds

Natural scaffolds are mainly hydrogels (colloidal gel, 99% water) made of natural materials or proteins like collagen type I, laminin or hyaluronic acid. These hydrogels are

Table 1 Engineered natural and synthetic scaffolds for 3D tumour studies

Type of scaffold	Cell line	Properties	References
Collagen	MCF-7 MYO1089 HB4a HMFU19	Type I collagen is the major ECM component of most tissues. Cell attachment to this matrix protein is via the $\alpha 2 \beta 1$ integrin. Stiffness of collagen scaffolds can be controlled for biomimetic tumour culture.	Holliday et al. 2009
Hyaluronic acid	C4-2B	HA is an integral component of the tumour ECM, activates many signalling pathways via CD44 and RHAMM Spontaneous cross-linking and cell encapsulation Allows for even diffusion of drugs	Gurski et al. 2009
Silk protein	MDA-MB-231 LNCaP	Biocompatible and porous scaffold Presence of RGD motif	Talukdar et al. 2011
PEG	OV-MZ-6 SKOV-3	Functionalization with RGD and MMP-sensitive site Can regulate stiffness by changing polymer dry mass without changing biological properties	Loessner et al. 2010
PLG	OSCC-3	Biocompatible and easy to reproduce Appropriate for co-cultures	Fischbach et al. 2007
PLGA/PLA microparticles	MCF-7	Surface modification via cross-linking with hydrophilic polymers or coating with ECM Formation of an internal matrix structure by sucrose leaching from the microparticles Porous matrix structure and high mechanical strength	Sahoo et al. 2005

mechanically weak, but provide the cells with a biological environment to proliferate in. The benefits of using hydrogels include: (1) the cells' ability to remodel the hydrogel, in particular collagen hydrogel, which allows the study of feedback loops in cell-matrix interactions; (2) an increase in matrix density through contraction; (3) the ability to apply uniaxial strain to hydrogels to generate alignment of cells (Cheema et al. 2003). A collagen hydrogel scaffold was used to construct an *in vitro* organotypic breast cancer model using three distinct cell types: luminal epithelial cells

(either normal or cancerous), myoepithelial cells, and fibroblasts derived either from normal or tumour stroma (Holliday et al. 2009). The authors demonstrated that the co-existence of the different cell types within the hydrogel resulted in cellular organisation that mimicked the *in situ* picture, with myoepithelial cells organising themselves around luminal epithelial foci. Interestingly, the addition of tumour stroma fibroblasts, unlike normal fibroblasts, disrupted the epithelial unit, as would be expected of a cancer focus escaping normal architecture to invade the surrounding ECM. This is one of the few studies describing a 3D *in vitro* model which uses *three* cell types, with other models using up to two cell types. The results demonstrate convincingly the pivotal role of tumour stroma fibroblasts in cancer progression.

Collagen hydrogels comprise a random mesh of collagen fibrils supporting a large amount of excess fluid (99%). A major drawback of such hydrogels is their very low density which does not mimic the naturally stiff environment in which tumour cells, and most tissue cells, naturally reside. Recently technologies to controllably increase density of this matrix have been developed, and resulted in more biomimetic scaffolds where only interstitial fluid from the hydrogel is lost, increasing the cell and collagen density (Brown et al. 2005). This technique of plastic compression (PC) can thus be used to control matrix density, resulting in densities of up to 30% collagen (Abou Neel et al. 2006). The stiffness of collagen scaffold affects cell growth and their morphology (Paszek et al. 2005). With increased stiffness, human tumourigenic mammary cell line

Fig. 2 A schematic diagram depicting (a) a 2D cell culture on plastic (b) a 3D cell culture in a porous scaffold; (c) *in vivo* tumour growth with supporting stromal cells

(HMT352 MECs) formed small growth-arrested and polarized acini with adherent junctions and central lumen. Therefore, matrix stiffness may modulate integrin adhesions and enhance malignant cell growth, but this has not been applied in a tumour 3D model to date.

One of the major features of an in situ tumours is the hypoxic core which drives angiogenesis to aid tumour growth (Harris 2002; Verbridge et al. 2010). Increasing collagen density in cell-seeded 3D scaffolds resulted in the formation of oxygen gradients, where human dermal fibroblasts on the surface were exposed to higher oxygen levels compared to the core. Fibroblasts within the core, where oxygen is low, up-regulated angiogenic factors, e.g., VEGF (Cheema et al. 2008). The level of hypoxia is easily controllable and dependent upon the matrix and cell density. Reversal of angiogenic upregulation, or ‘switching off’ of this signalling, is also possible through simple perfusion features, to mimic vasculature (Cheema et al. 2010). Such tissue engineering strategies could be employed to test cancer cell growth in these dense PC scaffolds, mimic the hypoxic core and investigate how cancer “recruits” a local blood supply. Using these techniques, increasingly complex 3D in vitro models can be built, but features such as addition of extra proteins, (including those of basement membrane) in appropriate proportions still need to be addressed.

Hyaluronic acid-based (hyaluronan, HA) hydrogels are another example of a natural scaffold. Hyaluronic acid is present in the ECM and is highly expressed in malignant tumours. HA hydrogels promote cancer cell proliferation and cluster formation (Gurski et al. 2009). This model was used to study the effects of heparin, which is the inhibitor of hyaluronidases (HAse; hyaluronan degrading enzymes), on tumour invasiveness of 13 human cancer cell lines (David et al. 2004). Heparin significantly inhibited invasiveness of HAse-producing cancer cell lines (brain and hepatic metastases). The HA hydrogel model was also used to test Camptothecin and Docetaxel, which are standard chemotherapeutics used in prostate cancer treatment, and Rapamycin, which is currently in clinical trials for different cancer types, including prostate (Gurski et al. 2009). This study demonstrated drug gradient action with cells on the outside of the prostate mass killed first, followed by the cells located inside. This is because drugs do not diffuse easily through the cancer cluster, as they do through the hydrogel.

Silk protein isolated from the silkworm *Antheraea mylitta* exhibits promising properties as a biomaterial (Mandal and Kundu 2007). Silk protein fibers have great tensile and mechanical strength and high elasticity. As scaffolds they form porous materials with improved swelling ratio and stability. Moreover, after treatment with 70% alcohol, proteins form β -sheets, which promote

scaffold stability in water and phosphate buffered saline (PBS) and confer in vitro resistance to degradation (Talukdar et al. 2011). Moreover, *A. mylitta* fibroin presents Arg-Gly-Asp (RGD) sequence within its structure. This is an integrin-binding motif intimately involved in cell attachment. Human breast cancer cells seeded within this scaffold showed good proliferation and migration through the porous material and formed clusters (Talukdar et al. 2011).

Synthetic scaffolds

Scaffolds can be made from synthetic polymers including polylactide (PLA), polyglycolide (PGA) and co-polymers (PLGA, PLG) which are biodegradable and form various structures, like mesh, fibers, sponge. They are designed specifically to mimic biomolecular structures present in vivo. Synthetic scaffolds are mechanically stronger than their natural counterparts, though cell adhesion to these polymers is much poorer. Thus, polymeric scaffolds have to undergo surface modification to improve cell adhesion. Recent advances allow for incorporating various functional groups at specific loci within the polymer chains (Cunliffe et al. 2004). Moreover, synthetic polymer functionalization can be also achieved by mixing scaffolds with specific ECM components (Chen et al. 2003). A number of studies have demonstrated the feasibility of using synthetic scaffolds for 3D cancer models as described below.

Polyethylene glycol (PEG) hydrogel can be functionalised with peptides (e.g., containing the RGD sequence) for enhanced cell-cell and cell-ECM interactions (Villanueva et al. 2009). PEG can be further cross-linked with MMP- or plasmin-sensitive peptide sequences to mimic specific degradability of the ECM (Raeber et al. 2005). Human epithelial ovarian cancer cells when encapsulated within a PEG-based hydrogel scaffold formed spheroids, structures which resemble the in vivo mass found in the peritoneal cavity of ovarian cancer patients with advanced disease. The same cells cultured in a 2D environment formed only monolayers (Loessner et al. 2010). Furthermore, altered matrix stiffness had an effect on the growth of cancer cell spheroids, with denser material causing slower proliferation and formation of small compact spheroids, whereas in soft hydrogels spheroids were irregular and scattered.

Co-polymers scaffolds are biocompatible, easy to reproduce and handle, and are amenable to large-scale use (Fischbach et al. 2007). Oral squamous cell carcinoma cells when cultured in PLG scaffolds formed tumour masses, with highly proliferative cells situated on the outside and apoptotic or necrotic cells towards the centre. Oxygen levels inside the tumour mass were similar to in vivo levels (15 ± 13 mmHg) (Fischbach et al. 2007). Moreover, the cells within the PLG scaffold released pro-angiogenic molecules

(VEGF, IL-8 and bFGF), corresponding to the hypoxic inner part of the tumour within the scaffold.

An alternative approach is the use of biodegradable, porous PLGA/PLA microparticles which can be modified by incorporating various materials, like chitosan or poly (vinyl alcohol) PVA (Sahoo et al. 2005). The microparticles offer the additional advantage of a large surface area which potentially supports cell adhesion and growth. Studies showed significantly better human breast cancer cell line (MCF-7) adhesion onto PLA microparticles with incorporated PVA. MCF-7 grew around the microparticles covering them and finally forming clusters. These results although preliminary support the further investigation into the usefulness of microparticles as a scaffold for tumour modelling.

Synthetic scaffolds have a great potential to mimic aspects of the natural microenvironment as they can be functionalized and designed according to requirements. However, cell behaviour will also depend on various properties of these biomaterials including their chemistry, material surface properties (hydrophobicity/hydrophilicity, topography) and structure (porosity, surface area, interconnectivity). Nevertheless, as synthetic scaffolds are simply not made of natural components, to achieve an in vivo-like structure, with all the essential microenvironmental cues, is a complex and challenging task.

Conclusion

Standard cell culture studies are widely used to delineate the biological, chemical and molecular cues of living cells, but as reductionist models they have limitations. The development of increasingly complex 3D in vitro models which aim to recapitulate the tumour microenvironment, in terms of cell types and acellular constituents will further our understanding of the cell-cell and cell-matrix interactions occurring during tumour growth and invasion. 3D studies demonstrate different cell morphology and expression compared to 2D, resembling more closely cells in vivo (Amatangelo et al. 2005) and affirming a role for 3D models in cancer investigations (Fischbach et al. 2007; Cukierman et al. 2001). The models explored so far give promising results: aggregation and clustering of cancer cells, migration and proliferation, release of angiogenic factors and formation of hypoxia within tumour masses. This further aids testing of the efficacy and molecular mechanisms of novel and existing drugs. However, 3D in vitro models also have limitations that need to be addressed. MCTS or ECM embedded models are still simplistic; they do not include all the cell types and acellular ECM components which would truly represent the desired tumour microenvironment and render the model

organotypic. Synthetic scaffolds are and will never be natural matrices. This is a barrier to mimicking the native tumour microenvironment and therefore synthetic scaffolds may be more useful for investigations of individual and specific tumourigenic steps. Utilizing tissue engineering techniques, 3D models may be carefully built up in complexity in terms of spatial positioning of different cell types, controlling matrix density and with appropriate matrix composition. Such improved biomimetic models present a bridge between 2D systems and in vivo models. Furthermore, they have the potential to provide a serious alternative to in vivo models, particularly in delineating molecular mechanisms underlying tumour growth and progression and drug action.

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