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Tissue Engineering & Modelling

Critical review

Cancer tissue engineering—new perspectives in understanding the biology of solid tumours—a critical review

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Abstract

Introduction

Understanding cancer biology is a major challenge of this century. The recent insight about carcinogenesis mechanisms, including the role exerted by the tumour microenvironment and cancer stem cells in chemoresistance, relapse and metastases, has made it self-evident that only new cancer models, with increased predictability, will allow the development of efficient therapies. The aims of this critical review are to briefly summarise and discuss the key aspects in the development of three-dimensional biomimetic tumour models. In this review, tissue engineering (TE) retains a valuable and highly exploitable potential. Tissue-engineered tumour models can account for a number of advantages, such as reproducibility, tailourable complexities (e.g., cell types, size, chemistry, architecture, mechanical properties, bioresorption and diffusion gradients) and ethical sustainability, making them suitable tools not only for mimicking normal tissue regeneration, but also, and most interestingly, for cancer development and resistance to therapies. Finally, we will focus upon interesting studies recently reported in the published literature about cancer TE, grouping their findings by tumour type, in order to give a snapshot picture of the current achievements to those cancer scientists, who are wishing to approach the field of TE. A special focus was given to pancreas, breast and prostate tumours.

Conclusion

There are marked intent affinities indicating TE as a suitable discipline to model cancer tissues. This is a topic of current efforts by several research groups worldwide, although, to date, well-defined guidelines have not been outlined yet, but rather preliminary individual studies have been reported.

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Introduction

Despite our body develops and evolves since the very first embryological events in a three-dimensional (3D) environment, nowadays we are still studying the processes at the base of developmental biology with a two-dimensional (2D) technology, i.e., with traditional *in vitro* cell cultures[1]. Extensive investigations have confirmed that cells change their phenotype when cultured in 2D conditions, which contribute to very long track, often decorated with unsatisfactory and contradictory results, characteristic of translating new medical therapies from the bench to the bedside[2]. Therefore, there is a tremendous need for new 3D cellular models enabling a thorough understanding of biological processes at the base of tissue and organ development, maturation, homeostasis and not to a lesser extent, degeneration and alteration[3]. The scientific community is still systematically using 2D models for drug screening[4]. There are a number of reasons that have consolidated this approach. Cancer cells are rapidly replicating and highly invasive, making their isolation and culture very simple. Because of the ease of handiness, the standardisation of cytotoxicity assays and later on, the association with computer-modelling tools for drug design, 2D cell cultures have thus become a widespread and accessible method for the preliminary assessment of tumour pharmacotherapy[5]. The other model widely used in cancer biology is typically an animal model in which human tumour cells are injected to form a tumour[6]. This method is very laborious and requires animal facilities as well as ethical approval. Both above-mentioned models suffer from important limits that can nullify the set-up of really effective therapies[7]. Intermediate 3D models have also been developed and handled by cancer scientists, known as spheroids and gel embedding, are able to mimic only limited aspects of tumour biology[8,9].

The concept of cancer TE is very recent, but holds great promise; indeed, convergences of objectives and methodologies between both disciplines have been highlighted and discussed elsewhere[10–12]. In 2006, at the dawn of cancer tissue engineering (TE) studies, the TE community pointed out their next-generation guidelines, underlining the necessity of complex biomimetic models, nicely correlating stem cell differentiation on TE scaffolds with developmental biology[13]. To achieve the formation of mature functional substitutes *ex vivo*, tissue engineers, were thus suggested to focus on the regeneration of metastable microenvironments, where complex cell-cell and cell-extracellular matrix (ECM) interactions can develop in a biomimetic fashion. Such guidelines

FOR CITATION PURPOSES: *Ricci C, Moroni L, Danti S.* Cancer tissue engineering—new perspectives in understanding the biology of solid tumours—a critical review. OA Tissue Engineering 2013 Apr 01;1(1):4.

Competing interests: none declared. Conflict of Interests: none declared.
All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.
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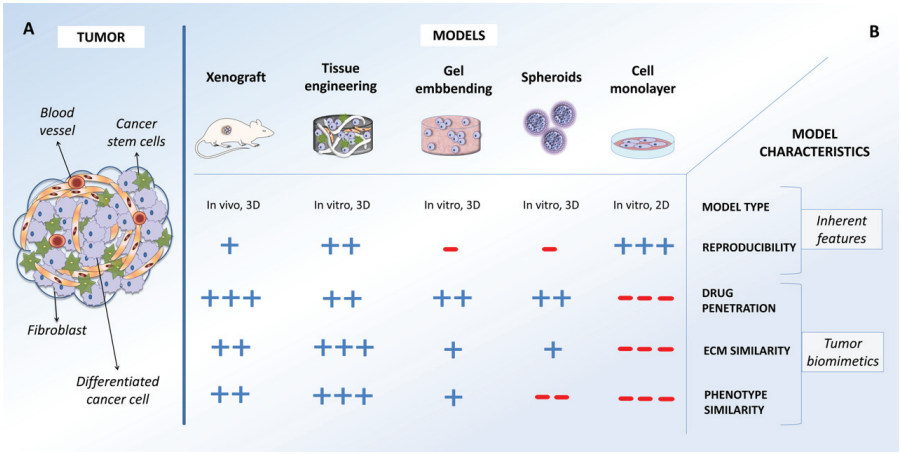

Figure 1: Schematic picture of (A) a tumour and (B) tumour models, with their main characteristics. Some important aspects were identified and qualitatively scored according to the findings of the published literature and to our personal experience. They include model-inherent features, such as model type and reproducibility, and some model biomimetic capabilities.
2D, two-dimensional; 3D, three-dimensional.

actually also retrace the features that an optimal tumour modelling should have. In this view, cancer development biology can meet the TE approach with a renewed emphasis. These new platforms can be exploited to learn about fundamental cell-biomaterial interactions and cell-cell communications, being valid for both normal and cancer cells. When cell populations are used to form tissues and organs, proper 3D systems, with clinically relevant dimensions, are required to eventually scale up these findings into effective new treatments[14].

In this critical review, we aim at collecting and discussing with educational intent, the key aspects involved in the design of new biomimetic cancer models, with a special focus on the role, potential and actual—so far—played by TE. Finally, the ultimate purpose of this critical review is to stimulate a propulsive interaction between cancer scientists and tissue engineers, to respond, via a highly multidisciplinary approach, to still unmet therapeutic needs.

Discussion

In this review, the authors have referenced some of their own studies. The protocols of these studies have been approved by the relevant ethics committees associated to the institution in which they were performed.

Tumour models: comprehension versus complexity

The search for cancer models has started in the second half of the last century and it is still in progress (Figure 1A–B). Traditional *in vitro* systems are 2D, but they offer the appealing advantage to the scientist, to be highly reproducible and responsive to drugs and radiations[6]. However, this model has revealed to suffer from a scarce predictability (Figure 1B)[7]. This is due to a number of reasons, whose deep understanding parallels the ongoing achievements in cancer biology, making 2D models insufficient. Basically, the lack of reliability seems to be associated to three main aspects as follows: cell sources, model dimensionality, and microenvironment complexity[7,12]. It has to be reminded that *in vitro* expansion and passaging of cells is known to produce phenotype selection and eventually, alteration with time[2,15]. This surely makes primary tumour cells preferable to long passaged and immortalised cell lines. However, beside mere cancer cells, as entities of action, the whole cancer microenvironment has recently shown a strong relevance in the comprehension of carcinogenesis and thus, in therapeutic success[16]. The tumour is a markedly variegated-3D tissue structure, comprising several cell types, exerting mutual support throughout the secretion of specific soluble factors and ECM molecules, including vascularisation (Figure 1A). Considering this, the very first cellular selection is performed during cell isolation from a tumour biopsy, as it involves native ECM disaggregation and culture selection of fast replicating and plastic-adaptive cells, to the detriment of cancer supporting cells. An additional concern related to cell source, which has been pointed out in the last few years, relies on cancer stem cells (CSCs) and their pivotal role in tumour eradication[17,18]. CSCs have been described as tumourigenic cells, which show stemness features, present in a tumour tissue at some concentration[18]. Such cells have been addressed as a distinct population of the cancerous tissue, but capable of long-term delivery of differentiated progenies of diverse cancer cell types. Therefore, CSCs have been invoked as the main cause of tumour relapse and metastasis[18]. In this respect, failure of traditional therapies could be explained with a wrong-cell targeting, because the differentiated cells are the most represented in tumours. Basic problem ever afflicting stem cell recognition and targeting, is the lack of specific surface antigens, which makes their direct identification usually tricky[19]. This is due to the undifferentiated nature of any stem cells and renders a panel of markers necessary to circumscribe, although not to strictly identify, the cell population of interest. On the other hand, sometimes differences between CSCs and normal stem cells have not been well-identified. Therefore, any CSC-targeted therapies are hypothesised to potentially affect normal

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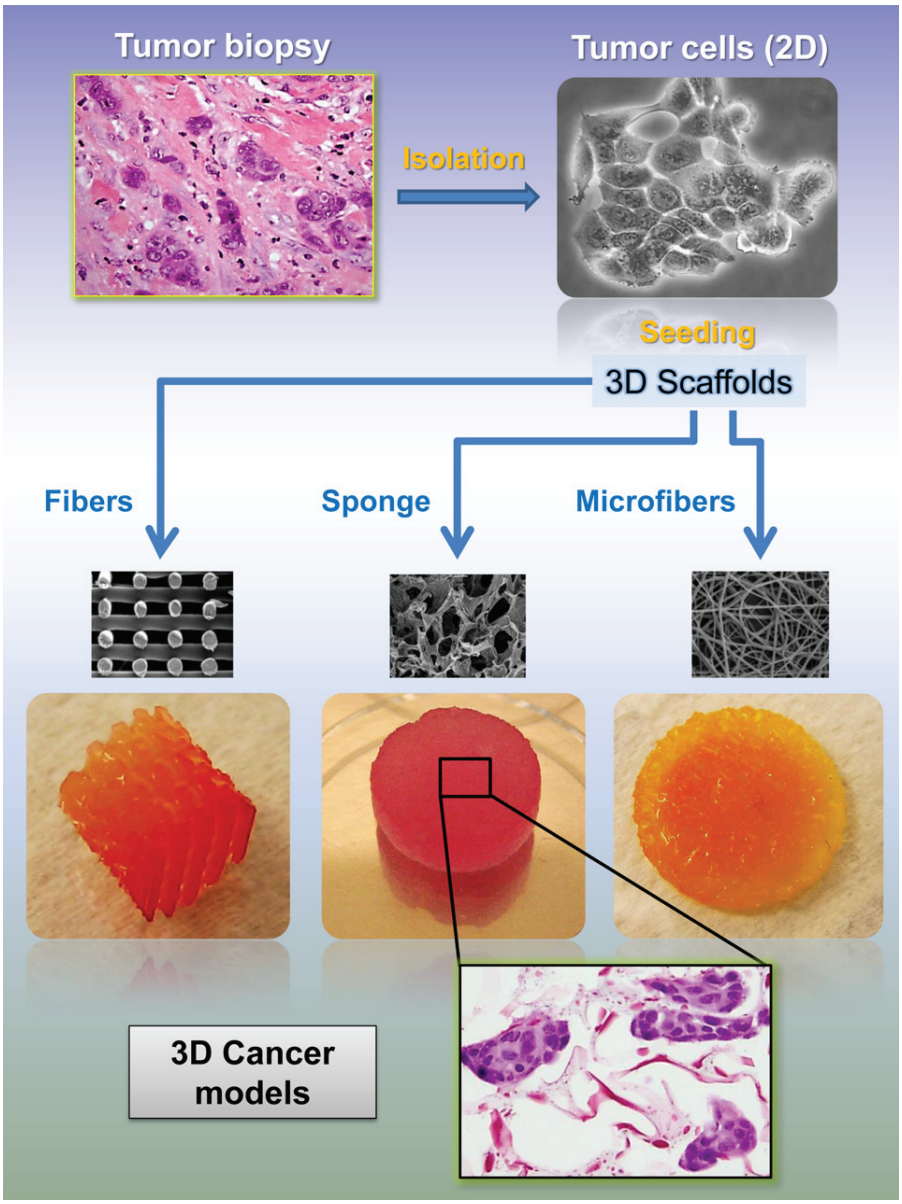

Figure 2: Flowsheet of TE cancer models. This example is rendered with images of a study related to human pancreatic ductal adencocarcinoma (hPDAC) performed in our laboratories. Consequentially, single images/image groups show the following: light micrograph of hPDAC morphology (haematoxylin and eosin stain); light micrograph of isolated primary hPDAC cells (PP244); scanning electron microscopy (SEM) micrograph of scaffold inner structures (3D fiber deposition via Bioplotter®, sponge via emulsion and freeze-drying, microfibers via electrospinning); hPDAC cell/scaffold constructs under different viability assays (colour gradients of the scaffold surfaces highlight spatial localisation of viable cells) and light micrograph of a spongy construct (haematoxylin and eosin stain) showing presence of 3D hPDAC cell clusters within the scaffold pores, whose morphology mimics that of native PDAC (see the tumour biopsy micrograph). 2D, two-dimensional; 3D, three-dimensional.

stem cells and to be detrimental for patients[20]. Although a CSC selection and 2D culture is possible for a therapeutic screening, other issues still remain unsolved. They include drug delivery, efficiency and selectivity, as well as tumour self-protecting mechanisms involving cell-cell and cell-ECM interactions. The understanding of all these characteristics involves the availability of complex tumour models able to contain diffusion gradients and to mimic the tumour microenvironment. We still need, essentially, biomimetic 3D models of cancer[1,3,10–12].

The most widely used 3D (complex) model of cancer biology is typically an animal model (Fig. 1B). *In vitro*-selected human cancer cells, are typically, injected in a nude animal as a host (xenograft) and grown to form tumour masses and metastases[6]. Although animal models have appeared very promising, they have resulted, in the end, as a poor predictive[7,12]. This can be explained with model-inherent reasons as follows: the immune system of the animals is compromised in order to host human cells, so it cannot be a part of the therapeutic screening, the life span of the animal (usually mice) is usually shorter than the relapse time of tumour in humans, and in the tumour microenvironment, the vascularisation and supporting cell infiltration (e.g., fibroblasts) are of animal origin, while the tumour cells are of human origin; this 'chimerism' can cause a completely unpredicted response to therapies[16]. To overcome these limits, advanced animal models, experimentally laborious, have been developed for some cancer types[12]. Nevertheless, there are still constitutive anomalies affecting the use of animals in the study of human diseases, such as ethics, cost-effectiveness and a general lack of predictability. However, presumably because of some anthropomorphic perception of the animal model, clinicians typically take a favourable look at the employment of *in vivo* tests for their research, and accept with difficulty, to put efforts for the improvement of *in vitro* models, which are conversely reproducible and ethically sustainable.

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Table 1. Tumour cell/biomaterial models for different cancer types.

Tumour type	Model	Biomaterials	Cell line; species	Main results	Year	Ref. #
Pancreas	TE	PVA + gelatine	PP244; human	Good growth and viability	2008	26
	TE	PGA-TMC + gelatine	isolated CSCs ($CD24^+$, $CD44^+$); human	Expression of cancer markers and cancer morphology	2013	27
	Gel	Fibronectin-gelatine	K643f, NIH3T3; murine	More biomimetic drug delivery and ECM	2013	25
	Spheroids	Methylcellulose	Panc-01, Capan-1 ASPC-1, BxPC-3; human	Improved chemoresistance with respect to 2D	2013	24
Breast	TE	Chitosan	MCF-7; human	3D growth conferred drug resistance	2005	31
	TE	PLA, PLGA	MCF-7; human	Tissue-like structure and drug resistance	2005	32
	TE	PLG + HA	MDA-MB231; human	HA improved cell adhesion	2010	29
	TE	PLG + HA	MDA-MB231; human	Good proliferation	2011	30
Prostate	TE	PCL-TCP	PC3, LNCaP; human	Increased invasion potential	2010	34
	Gel	PEG-Gln/PEG-MMP-Ly	LNCaP; human	Upregulated expression of MMPs, steroidogenic enzymes, and prostate specific antigen	2012	35
Oral	TE	PLG	LLC, MCF-7, U87; human	Tumour-similar ECM and hypoxic condition in 3D model	2007	1
Colorectal	Gel	lrECM/matrigel	CACO-2, COLO-206F, DLD-1, HT-29 SW-480 COLO-205; human	Different morphology from metastasis and primary cells	2013	36
Lymphoma	TE	PS	Z138, HBL2; human	Higher growth in 3D	2013	37
Lung	Spheroids	AlgiMatrix™	NSCLC cell lines (H460, A549, H1650, H1650 stem cells); human	Higher resistance to anticancer drugs than 2D (increased IC_{50} values of drug and reduced cleaved caspase-3 expression)	2013	38
Ewing Sarcoma	TE	Electrospun PCL	TC-71; human	Tumour biomimetics of morphology, growth kinetics and protein expression profile	2013	39

2D, two-dimensional; 3D, three-dimensional; CSC, cancer stem cell; ECM, extracellular matrix; HA, hydroxyapatite; MMPs, matrix metalloproteinases; PCL-TCP, polycaprolactone-tricalcium phosphate; PCL, polycaprolactone; PEG, polyethylene glycol; PGA-TMC, poly(glycolide-co-trimethylene carbonate); PLGA, poly lactic-co-glycolic acid; PLG, poly(lactide-co-glycolide); PVA, poly(vinyl alcohol); PS, polystyrene; TE, tissue engineered.

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In time, *in vitro* models of cancer have started evolving towards the third dimension[8,9,21]. Simple 3D *in vitro* models used by scientists include spheroid formation and gel (usually collagen-derived) embedding of tumour cells (Figure 1B)[9]. Spheroids are culture artefacts leading, for some transformed-cell types, to an induced cell aggregation in the form of compact spheres with diameters ranging from 20–1,000 μm[8]. For their nature, spheroids partially mimic the tumour microenvironment as follows: they show secretion of tumour ECM, 3D cell-cell interactions, diffusion gradients and increased chemoresistance, while phenotype diversity is missing[12]. Moreover, spheroid-based assays generally lack accuracy due to several difficulties in the management of these cell aggregates. With the attempt to improve 3D models, cancer cells have also been embedded in biologic hydrogels, which should mimic the primary ECM of tissues. However, such gels usually show insufficient porosity to obtain long-term cell survival and proper tumour ECM deposition. Moreover, spatial distribution of cells in the gel is often not uniform, thus resulting in poor consistent models[9].

Recently, microfluidics circuits have been developed to make a further step towards 3D cultures in cancer[22]. Yet, when macroscopically relevant dimensions (higher than 1 mm^3) are achieved, nutrient diffusions and cell survival remain problematic[14]. To solve these challenges, microfluidic well systems, with the capacity of controlling nutrient perfusion, have been developed and used alone or in combination with hydrogels[22].

Different from xenograft, spheroids and gel embedding, TE models can potentially offer all the fundamental achievements to cancer studies obtained so far for the regeneration of normal tissues as follows high standardisation of assays, multiple cell-type interaction, tailourable architecture allowing spontaneous 3D cell disposition and ECM synthesis, mechanical properties matching those of the tissue and tuneable diffusion profiles, thus appearing, in the end, as potentially elective models for the regeneration of 3D tumours (Figure 1B)[1,3,10–12,23].

Engineered tumours: achievements and perspective

A TE model of cancer should be a bottom-up 3D reconstruction of the tissue, using selected cells (CSCs or tumour cell mixtures), derived from primary cultures or from tissues, thus retracing the schematic diagram shown in Figure 2[23]. For each tumour type, suitable scaffold architecture should be identified, ideally which is able to match the topographic and mechanical aspects of the native tissues[1,3,10–12,23].

The current state-of-the-art about the development of *in vitro* 3D-biomimetic model for some important tumours is reported in Table 1. An overview was given of relevant studies involving the interaction of biomaterials and tumour cells to generate 3D cancerous constructs *in vitro*[1,24–39]. A special focus was finally given to pancreatic, breast and prostate cancers, as such topics already account for a number of published studies about the 3D interaction of cancer cells and biomaterials.

Pancreas cancer models

Due to its inauspicious prognosis, pancreatic ductal adenocarcinoma (PDAC) is the object of persistent studies. The development of an *in vitro* 3D model that simulates the specific PDAC microenvironment remains an important goal to be achieved in order to develop efficient therapies. In a recent study, various cell lines of pancreatic cancer (Panc-01, Capan-1 and ASPC-1) were used to form spheroid structures embedded in methylcellulose[24]. In the 3D model, gene expression profiles and ECM components were upregulated, while inhibition of selective microribonucleic acids (miRNAs) demonstrated an enhanced chemoresistance. A gel embedding-like approach has been recently reported by Hosoya and colleagues[25]. The proposed 3D model is created on Transwell® inserts alternating layers of gelatine-fibronectin and cells, thus reproducing some of the basic ECM structural features. This model was set up to study the diffusion of dextran nanoparticles using a murine fibroblast cell line derived from pancreatic tumour and normal fibroblasts as controls. With tumour-derived cells, results showed a decreased permeability of the dextran depending on the layer number and nanoparticle size demonstrating a good similarity with the tumour ECM. In this critical review, we discuss on a couple of studies, which reported about a TE approach for PDAC study[26,27]. Both groups employed scaffolds based on synthetic polymers, with defined architecture and surface morphology, to regenerate the PDAC in combination with gelatine to ensure cell adhesion and growth. In the first study, the human PDAC (hPDAC) cells, PP244, were grown on polyvinyl alcohol (PVA)/gelatine sponges, and cell metabolic activity was compared with that obtained in classic 2D culture controls[26]. The results showed viable cells, with enhanced metabolism, in the 3D model. The second and most recent study used CSCs, derived from human pancreatic tumours, showing $CD24^+$ and $CD44^+$, grown on poly(glycolide-co-trimethylene carbonate) (PGA-TMC) scaffolds[27]. In this critical review, the 3D model displayed an improved neoplastic formation, with tumour volume and weight higher than those of the 2D model. Such findings also confirm the TE-model validity for the expression of pancreatic cancer markers, such as the carbohydrate antigen 19-9 (CA 19-9), epidermal growth factor and myosin-1B (MIB-1).

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Breast and prostate cancer models

The 3D models have been developed to study metastasis initiation and development, with the use of cellular aggregates or spheroids, and microfluidic devices[22,23]. Considering the relevance of breast and prostate cancer mortality due to their metastasis to bone, 3D models derived from TE know-how, have been developed to study metastatic events of these cancer types to bone engineered tissue. Cancer cell angiogenic signalling was regulated by integrin and correlated with enhanced production of interleukin-8 (IL-8). Further control over tumour angiogenesis was influenced by oxygen availability in 3D tumour culture models, with increased levels of IL-8 secretion in normoxia and of vascular endothelial growth factor in hypoxic culture conditions[28]. Similarly, porous biomaterials containing inorganic phases like hydroxyapatite (HA) were used to create initial models of breast metastasis into bones and revealed a role of HA crystal size in tumour cell adhesion and proliferation[29,30].

Basic 3D systems have shown that breast and prostate cancer cells, among others, are indeed more resistant to chemotherapies than when cultured on 2D substrates, thus justifying the continued development of advanced *in vitro* models that can replicate not only cell-cell communication as in current spheroid models, but also cell-ECM interactions[31–33]. Spheroid and microfluidic culture systems are constrained to very small artificial environments in the order of few hundreds of microns, which fail to recapitulate the heterogeneous complexity of bone tissue and prostate metastatic niches. The collaborative efforts of Hutmacher's and Clement's groups have also demonstrated that 3D scaffolds can be used to study events at the base of bone metastases, which showed increased invasion potential and upregulated expression of matrix metalloproteases, steroidogenic enzymes and prostate specific antigen[11,34,35].

Conclusion

There are marked intent affinities indicating TE as a suitable discipline to model cancer tissues. This is a topic of current efforts by several research groups worldwide, although, to date, well-defined guidelines have not been outlined yet, but rather preliminary individual studies have been reported. Recent studies have reinforced the theoretical hypothesis that tissue-engineered cancer constructs can mimic the tumour microenvironment because of their three-dimensionality and their multi-parametric tailourability. The interactions between tumour cells and different biomaterials seem to play a key role in tumour biomimetics to be finely exploited in the very near future.

Abbreviations list

2D, two-dimensional; 3D, three-dimensional; CSC, cancer stem cells; ECM, extracellular matrix; HA, hydroxyapatite; hPDAC, human PDAC; IL-8, interleukin-8; PDAC, pancreatic ductal adenocarcinoma.

Acknowledgements

Authors wish to acknowledge Dr. Niccola Funel and all members of the Anatomical Pathology Unit of Cisanello Hospital (AOUP, Pisa, Italy) for experimental and theoretical support on pancreas cancer.

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FOR CITATION PURPOSES: *Ricci C, Moroni L, Danti S.* Cancer tissue engineering—new perspectives in understanding the biology of solid tumours—a critical review. OA Tissue Engineering 2013 Apr 01;1(1):4.

Competing interests: none declared. Conflict of Interests: none declared.
All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.