Biomaterials offer cancer research the third dimension

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To deepen understanding and hasten the development of treatments, cancer needs to be modelled more accurately *in vitro*; applying tissue-engineering concepts and approaches in this field could bridge the gap between two-dimensional studies and *in vivo* animal models.

In a mini review from 2002, Tyler Jacks and Robert Weinberg[1] commented on the pioneering three-dimensional (3D) culture work from Bissell laboratories[2] and concluded: "Suddenly the study of cancer cells in two dimensions seems quaint if not archaic." The relevance of this statement for planning and executing mechanistic biological studies and advanced drug testing has been largely disregarded by both academic researchers and the pharmaceutical and biomedical industry in the twenty-first century. As a result, a Medline search shows that surprisingly more than 70–80% of cancer and molecular biologists still use two-dimensional (2D) techniques in their work, namely methods such as tissue-culture plates, Petri dishes, cover slips and so on.

Studies in standard cell culture have produced many results to help us to interpret complex biological phenomena and hypotheses. However, from an anatomical and physiological point of view, cancer cells cultured in three dimensions are characterized by several factors differentiating them from monolayer cultures and paralleling much more closely those of *in vivo* tumours. In particular, early events of tumour growth before effective vascularization appear to be closely reproduced in those 3D culture systems. Usually, 3D cultures of tumour cells develop hollow cores that resemble the necrotic areas of *in vivo* cancers: areas that are usually observed at a distance from nutrient and oxygen supplies. Importantly, the proliferation of tumour cells cultured in three dimensions is typically slower and hence more reminiscent of physiological growth than that of monolayer cultures.

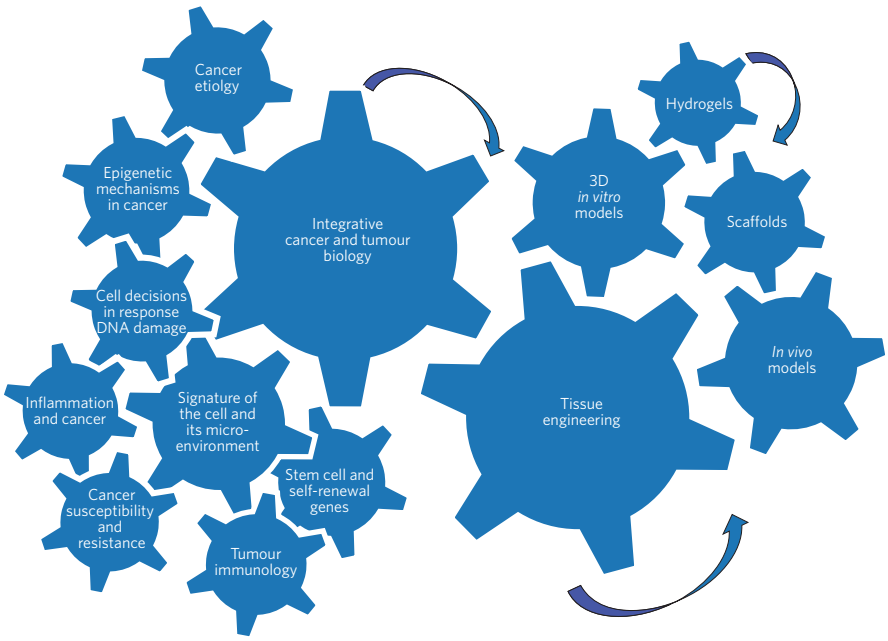

Figure 1 | Graphical illustration of how technology platforms originally developed for tissue-engineering applications produce valuable models that mimic 3D tissue organization and function by replicating physiological and pathological conditions of cancer as close as possible. In 2003, the Division of Cancer Biology of the National Cancer Institute was asked to create a series of 'think tanks' to assess the state of cancer biology research and to recommend to the National Cancer Institute a research agenda that would accelerate progress in cancer research. It is stated in the report (http://www.nci.nih.gov/think-tanks-cancer-biology/page3) that achieving the overall goals can be expedited by encouraging interdisciplinary research teams and multi-institutional collaborations, and that advances in technologies that have been identified as critical are new *in vitro* 3D matrix reconstitution and organotypic models, and animal models.

Advances in tissue engineering have traditionally focused on the design of scaffold- or matrix-based culture systems and models that reflect as closely as possible the biological, physical and biochemical environment of the natural extracellular matrix (ECM). Although clinical applications based on tissue-engineering concepts such as the replacement of body tissues attract most of the media attention, it is apparent that other fields of medical research could be enhanced by the powerful and modular tools already developed in tissue engineering. For example, 3D *in vitro* and/or *in vivo* tissue-engineering models that are designed to resemble the physiology of tissues could be used to study disease pathogenesis of tumours[3,4]. Here, we take a look at the role that biomaterials originally developed for tissue-engineering platforms could contribute to future cancer research, particularly with 3D *in vitro* and *in vivo* tumour modelling (Fig. 1).

The *Nature News* feature in 2003, 'Biology's new dimension' and the accompanying editorial, 'Goodbye flat biology', were timely and highly valued

by the small number of researchers and scientists already working on developing 3D culture models[5]. These selective groups emphasized the basic necessity for study within 3D culture systems before turning to whole-animal studies for therapeutics development, as well as for basic research in tumour biology. This article also featured a prediction by one of the chief scientific officers of a large pharmaceutical company: "In 10 years, anyone trying to use 2D analyses to get relevant and novel biological information will find it difficult to get funded." However, that prophecy was not fulfilled as most of the funded *in vitro* work in the twenty-first century is still performed in two dimensions[6].

Attaining accurate cell-culture information for *in vivo* prediction is important in cancer research to ensure efforts and funding are focused towards the most promising research channels. To help achieve this, the National Cancer Institute (http://www.cancer.gov) announced in 2002 the start of a new US$40-million-per-year programme titled 'Signatures of the Cancer Cell and its Microenvironment', designed to investigate the impact of the microenvironment on tumour-cell behaviour. This initiative emphasized in its grant call that it was seeking grant applications in respect to 3D culture technologies, and it was hoped that this would clearly foster the acceptance of such approaches.

Undoubtedly, starting with this programme, a small shift has occurred in both the cancer-research community and funding agencies that support tumour biology as to the relative importance of microenvironmental control in cancer research. However, by reviewing the present cancer literature one might conclude that the general understanding and appreciation of the complexity and the extent of the microenvironment's influence on tissue function and dysfunction is still limited and at times simplistic, as most cancer researchers still use predominantly a 2D culture system. One might hypothesize that this is one of the reasons why we have failed to see significant breakthroughs over the past 10 years in cancer-drug development as well as new and innovative treatment concepts.

Tissue-engineering principles

Originally articulated for the foundation of architecture theory, the axiom of 'form follows function' was also applied by tissue engineers at the start of this research field in the 1980s, only the other way around, namely that 'function follows form'. Right from the beginning of tissue-engineering research, 3D culture was important for innovation in the field and sparked the design and development of scaffold- and matrix-based technology platforms.

In 2003, the National Science Foundation published a comprehensive report titled 'The Emergence of Tissue Engineering as a Research Field' (http://www.nsf.gov/pubs/2004/nsf0450/start.htm), which summarizes chronologically the history of tissue engineering. Skalak *et al.* in 1988 were the first to define tissue engineering from a broad and general perspective as "the application of the principles and methods of engineering and life sciences towards the fundamental understanding of structure–function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain, or improve functions." Originally, tissue engineering aimed to mainly develop strategies in support of the clinical domain. However, although less heralded in the twenty-first century than the direct clinical applications, technology platforms originally developed for tissue-engineering applications are emerging as a powerful toolbox in other biomedical research areas, such as cancer, immunology and virology[7,8].

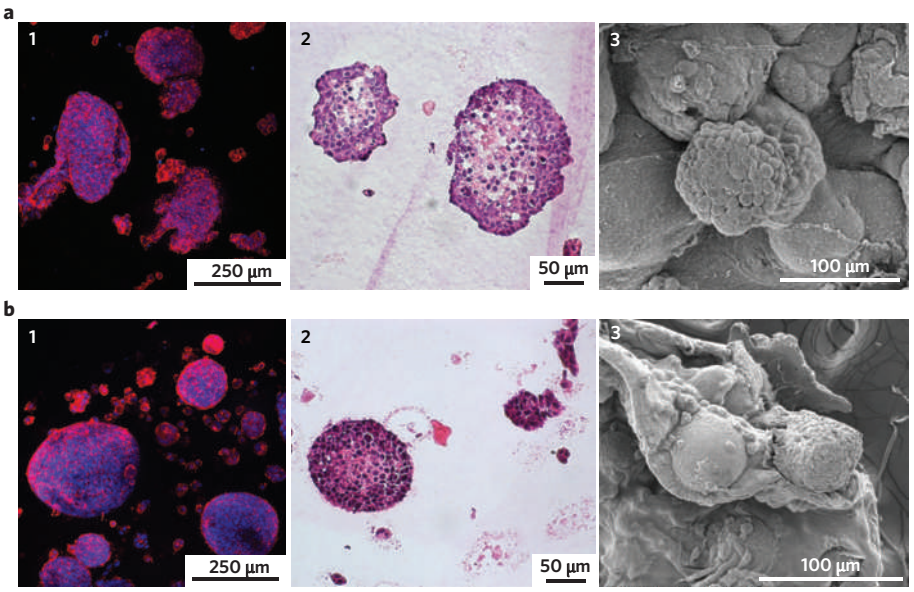

Figure 2 | Comparison of a synthetic PEG-based platform (**a**) with a hydrogel made of rat collagen I (**b**) in the formation of spheroid structures by culturing prostate-cancer cells (LNCaP). The design flexibility of biochemical and biological characteristics of these PEG-based hydrogels, combined with the high fabrication reproducibility, might make them superior to collagen and other natural hydrogels. This enables the influence of incorporated biomolecules and/or protease substrates on the behaviour of cells cultured in 3D to be studied. Both hydrogels show colony formation over the first week of culture; and subsequently spheroid structures combined with ascites after 21 days as shown by confocal laser microscopy (**a**1, **b**1) histology (**a**2, **b**2) and scanning electron microscopy (**a**3, **b**3). Next to the fact that collagen gels do not have a high design flexibility, the biomimetic PEG gel did not lose size and shape whereas the collagen gel showed significant shrinkage in the long-term culture.

Today, one of the principal outcomes of tissue engineering is that the functional properties of cells can be observed and manipulated in 3D culture platforms to an extent that is not possible in animal experiments. One can therefore conclude that after two decades, 3D culturing using scaffolds (porous cellular solids) and matrices (hydrogels) has played a key part in the advancement of tissue engineering and regenerative medicine[8].

Scaffold- and matrix-based models

Biomaterials of natural origin, with laminin-rich extracellular matrix (lrECM)[9] being the most prominent one, but also collagen gels[10], have been used by some cancer-research groups for more than two decades. Although these biomaterials have similar micro- and nanolength dimensions of the fibril native ECM, their main drawback is that they often contain residual growth factors, undefined constituents or non-quantified substances, and batch-to-batch variations make it difficult to compare and correlate work from different groups[10,11].

Hence, to advance the field further, reproducible 3D cell-culture systems for cancer research are very desirable,

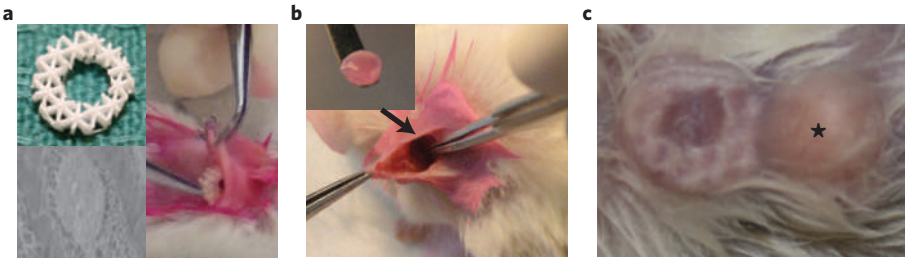

Figure 3 | The author's interdisciplinary group is at present creating an 'all human' model in which tissue-engineered human bone is transplanted into immunodeficient (NOD/SCID) mice and compared to the standard bone-chip model to study bone metastases related to prostate and breast cancer. **a**, *In vitro* bone engineering by using mPCL-TCP scaffolds (shown upper left) in combination with primary human osteoblasts (shown lower left). Implantation of tissue-engineered construct into the flanks of NOD/SCID mice (note that the scaffold/cell construct has a geometry similar to long bones with a hollow core that mimics bone-marrow cavity). **b**, Eight weeks after implantation of the tissue-engineered construct, second surgery is performed to implant a biomimetic hydrogel (shown upper left and see also Fig. 2) seeded with breast-cancer cells. **c**, Mice then develop reproducibly seized tumours next to the tissue-engineered bone eight weeks after the second surgery, as indicated by the star symbol.

particularly if they can be produced on a large scale from either natural, synthetic or hybrid biomaterials with well-defined constituents. Although the molecular composition of the ECM is a well-known regulator of cellular responses, the physical properties of the matrix in 3D models can also have surprisingly important roles. In particular, recent evidence points to direct roles for the stiffness of the ECM in regulating multiple cellular functions[12]. Also described as rigidity, elasticity or pliability, this property is sensed by cells through bidirectional interaction with the surrounding ECM. Cell-surface integrin receptors and the contractile cytoskeleton pull against the ECM to sense the stiffness of the microenvironment. Biologically, cells do sense and respond appropriately to their local ECM. The stiffness of microenvironments is highly variable: for example, it is manifest as loose versus dense connective tissue; soft (liver, kidney, skin, lung and so on) versus hard tissues (cancellous and cortical bone, teeth); and early versus late stages of tumour and metastases development. Therefore the capability to control the mechanical properties of scaffolds and hydrogel systems allow us to investigate whether different cancer populations of tumour cells in 3D structures might favour a soft or harder environment.

Tissue engineers have focused on developing scaffolds and matrices that can mimic key features of ECM, and at the same time provide the possibility of flexibly altering their physical and biochemical characteristics. Well-defined and characterized biomaterials-based tissue-engineering platforms are appealing because they offer batch-to-batch uniformity with improved, controllable and reproducible architecture, degradation rates and mechanical properties. Here, biopolymers may offer a suitable alternative to overcome the present limitations in cancer research.

Recent advances in biomaterials research have enabled engineering of hydrogels to include structure and function found in the natural ECM and allow the design and execution of systematic studies in cancer biology. For instance, functionalized alginate gels were used as 3D *in vitro* models to specifically explore the implications of the engagement of tumour-cell integrins in angiogenic signalling[13]. Commercially available Extragel, consisting of chemically modified hyaluronan and gelatin crosslinked with polyethylene glycol (PEG), have potential as tunable 3D cell-culture matrices in cancer-cell research[14]. These matrices have already been used as delivery vehicles for tumour cells for the creation of orthotopic human tumour xenografts in animal models.

The Stupp laboratories[15] reported the discovery of a self-assembling peptide system that can undergo spontaneous physical crosslinking into hydrogels by alteration of salt concentration at physiological pH. Structurally, these peptide-based synthetic hydrogels resemble the ECM and, if desired, can also incorporate bioactive peptides to initiate cellular responses. These matrices have been applied in a range of *in vitro* and *in vivo* studies, and the commercially available Puramatrix, originally developed in Zhang laboratories[16], have also been used as 3D cell-culture matrices in cancer research. Another synthetic hydrogel system that is versatile in terms of modular design and biological, biochemical and mechanical properties has been pioneered by Hubbell, Lutolf and co-workers[17] and is used in collaboration by the authors group to develop 3D cancer models (Fig. 2).

Mooney's group[13] showed that scaffolds made from a biodegradable polymer approved by the Federal Drug Agency were used for culturing human oral squamous carcinoma cells and gave rise to tumour-like masses with characteristics that, in contrast to monolayer culture and to some extent to cells cultured within the present gold-standard matrigel, expressed a very similar behaviour observed in animal models. Kaplan and co-workers[18] as well as my group (Fig. 3) used their independently developed scaffold-based bone-engineering platforms to develop an *in vivo* bone metastases model.

Every one of these studies show that 3D tissue-engineering matrices can be put to use with the aim of modelling cancer *in vitro* before moving into animal studies.

'To walk the talk' is the key to success

We are often informed that the most effective approach to interdisciplinary research is to build connections with experts in different fields and start collaborations. However, the scale and complexity of a biomedical problem such as cancer demands that everybody moves beyond the confines of their own discipline and work together in synergistic teams. It should, however, be kept in mind that despite the opportunities, there are clear differences in philosophy, approach, expectations and language between cancer researchers and tissue engineers that can make it very difficult to build successful collaborations, especially if the groups are not working at the same institute.

The David H. Koch Institute for Integrative Cancer Research at the Massachusetts Institute of Technology (http://web.mit.edu/ki/about/index.html) is a very good example of how success can be found if the interactions of the diverse and multidisciplinary groups are consolidated under one roof. This type of model institute has not only created a series of new research directions but also has the vision to train a new generation of PhD students and post docs capable of tackling interdisciplinary problems — 'to walk the talk' right from the beginning of their careers.

Interdisciplinary innovation in cancer research is becoming apparent throughout the world. In Australia, Queensland University of Technology's reputation for groundbreaking and multidisciplinary

prostate-cancer research has been recognized with federal government funding for the establishment of a world-class multidisciplinary research facility. The Australian Prostate Cancer Research Centre — Queensland will initially be housed at the Prince Alexandra Hospital, but will ultimately move to the $300 million Translational Research Institute, which is due to open in Brisbane in 2012. To meet the vision and objectives of the centre, the aim is to not only optimize cancer-research platforms but also transform clinical practice and allow investigators to reach their zenith by fostering innovation in investigator-initiated research and expanding access to resources, tools and technologies.

For my own research group, we have established dynamic collaborations with a number of cancer groups within the university. We worked with several groups to explore the potential of bone-engineering technology platforms with an initial focus on unlocking some of the mechanism of bone metastases (Fig. 3). Documented collaboration, such as joint publications, abstracts, PhD-student exchange and co-supervision, is also critical for the success of a grant application as the review panel always pays close attention to this aspect of the proposed interdisciplinary work. As we could fulfil the above criteria I was successful in securing a grant from the Australian Prostate Cancer Foundation, which has a specific funding scheme to attract researchers originally rooted outside the cancer field.

It is not projected that 3D culture models can entirely reproduce the colossal complexity of tumour biology and metastases formation. As a result, it is unlikely that they will ever be able to completely replace *in vivo* models in the analysis of the molecular mechanisms underlying the different types of cancer. But by breaking down the associated physiological and pathological complexity into an experimentally amenable number of distinct interactions, innovative 3D models could help bridge the gap between traditional 2D cell-culture methods and expensive and labour-intensive animal models. ❐

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Acknowledgements

D. W. H. thanks his Post Docs and PhD students S. Rizzi, S. Sieh, V. Reichert, P. Hesami and S. Siamadezh, and the Prostate Cancer Foundation Australia for supporting his research.