Continuing Professional Development

CPD

AUTHORS:

Biosketch - Dr Marie McIlroy is a Lecturer within the Department of Surgery at RCSI University of Medicine and Health Sciences and member of the Endocrine Oncology Research Group.

My research is focused on understanding how tumours utilise bioavailable steriods and the implications this may have on the development of resistance to endocrine therapy. An unsustained response to therapy is a major problem in the treatment of breast cancer, impacting around a third of hormone receptor positive patients. Although there have been advances in the past few decades with regards newer drugs including aromatase inhibitor (AI) therapy, a complete understanding of the mechanisms of resistance to these drugs and how it may be circumvented still evades us. How breast cancer cells adapt to an altered steroid state is unclear and we aim to address this using a combination of proteo-genomic analysis in the translational setting.

Professor Leonie Young leads the Endocrine Oncology Research Group based at York House in the Royal college of Surgeons in Ireland. Leonie's research focuses on breast cancer cell adaptation on metastatic progression, with a particular focus on brain metastasis.

60 Second Summary

The inception of cell culture techniques in the 19th and early 20th centuries changed the way we conduct research and allowed scientists to study live cells and tissues in ways that drove forward our understanding of much of basic cell biology.

Historically cancer cell line models have been the workhorse for the identification of new compounds with anti-cancer potential. One of the first serially transplanted PDX was developed in the 1970's and was reported to exhibit a consistent histological and karyotypic profile to that of the original tumour.

Organoids provide a physiologically relevant alternative to PDX, retaining the 3D architecture of the original tumour whilst avoiding mouse host-driven evolution.

Bio-printing is the spatially controlled positioning of biological materials, living cells and bio-chemicals to produce structures that are biologically functional and mimic the in vivo environment. This technology has been utilised in the area of regenerative medicine for a while now and there is increasing interest in using it as a therapeutic screening strategy for cancers.

The belief that simply inhibiting cell proliferation would prove to be the Achilles heel of tumour cell immortality has been challenged over the past few decades.

1. REFLECT - Before reading this module, consider the following: Will this clinical area be relevant to my practice?

2. IDENTIFY - If the answer is no, I may still be interested in the area but the article may not contribute towards my continuing professional development (CPD). If the answer is yes, I should identify any knowledge gaps in the clinical area.

3. PLAN - If I have identified a knowledge gap - will this article satisfy those needs - or will more reading be required?

4. EVALUATE - Did this article meet my learning needs - and how has my practise changed as a result?Have I identified further learning needs?

5. WHAT NEXT - At this time you may like to record your learning for future use or assessment. Follow the 4 previous steps, log and record your findings.

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Advances in 3D patient-derived cancer models; new drug screening approaches for breast cancer.

Introduction

The development of patient derived organoids and xenograft models have enhanced our approach to drug screening bringing in the highly valued element of personalisation. Technological advancements in the field of organoid culture and 3D-bioprinting have transformed our ability to model patient tumours and facilitates the layering of multi-omics data and drug sensitivity via high-content screening that has previously been unfeasible.

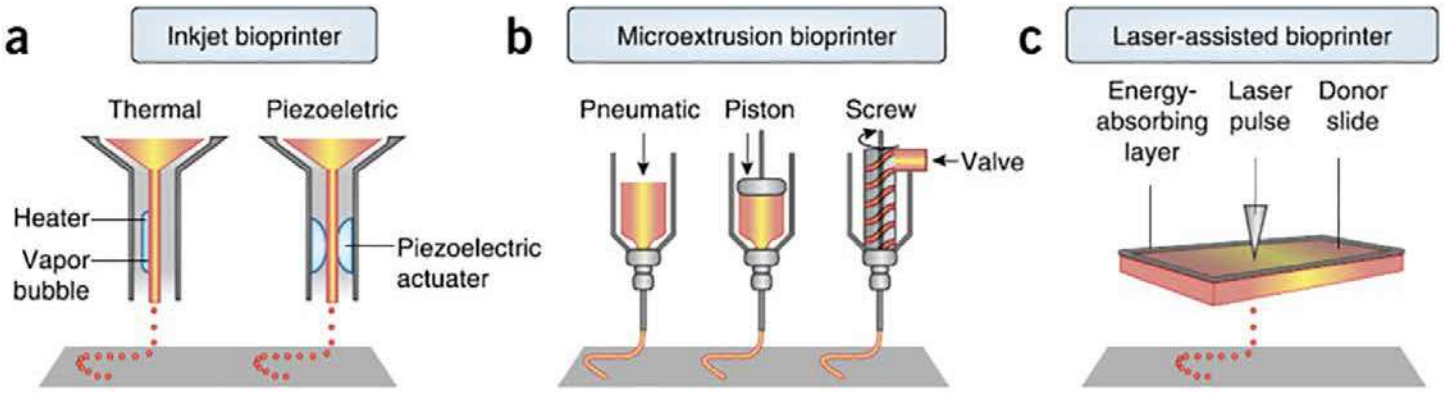
How 2D culture fell flat

The inception of cell culture techniques in the 19th and early 20th centuries changed the way we conduct research and allowed scientists to study live cells and tissues in ways that drove forward our understanding of much of basic cell biology.[1] It was however the work of George Gay and the development of the immortal HeLa human cervical cancer cell line that revolutionised the field of cancer research and it is a testament to their impact that they are still routinely used by labs worldwide today.[2] Despite the great advances achieved through the cell-culture techniques developed and refined from the 1950's there was a growing awareness amongst the research community that there were major drawbacks to this approach. Firstly, cells in our bodies do not grow in 2- dimensions and secondly, organs and tumours are comprised of complex, multi-cellular interactions between epithelial, mesenchymal and vascular elements. Clearly this was an area that required an upgrade and the past 10 years has heralded 3D cancer cell modelling as the norm rather than the exception.

The problem – disconnect between pre-clinical screening and clinical trial performance

Historically cancer cell line models have been the workhorse for the identification of new compounds with anti-cancer potential. Initial screens in the mid-20th century utilised transplantable murine leukemias, P388 and L1210[3] which despite obvious limitations were very successful in identifying potent chemotherapeutic agents. The development of the NCI-60 cell line panel in the 1980's introduced a much needed human element to the screening process and led to the identification of a number of drugs with efficacy against specific cancer types including Bortezomib (multiple myeloma), eribulin (breast cancer).[4, 5] The use of human cell lines in cancer screening also threw up some unanticipated paradoxes with a huge degree of variability in drug sensitivity across cancer cells from specific cancer sites, undeniably due to the molecular drivers of each individual tumour. Whilst 2D cell panels and their *in vivo* transplantation yielded promising pre-clinical data this very often did not translate when tested in a clinical setting. Indeed, the clearest indication that 2D cell culture provides limited data on drug sensitivity at a population level is the poor performance of lead compounds that enter clinical trial. In fact, just over 50% of compounds fail to progress in Phase II-III clinical trial.[6] This poses huge problems not only for the Pharma companies who

Sean V Murphy & Anthony Atala, Nature Biotechnology



have invested heavily to bring the lead compounds to this stage in development but it is also an inadequate outcome for patients who have enrolled in these studies, often accompanied by side-effects which negatively impact their quality of life.

Patient derived xenografts

Patient derived xenografts (PDX) are singly or serially transplanted tissues/ cells derived from either primary or metastatic origin (effluent, ascites, circulating tumour cells). One of the first serially transplanted PDX was developed in the 1970's[7] and was reported to exhibit a consistent histological and karyotypic profile to that of the original tumour. PDX have been utilised as screening tools in Phase II clinical trials of chemotherapy agents in the past, and in recent years have gained popularity in determining therapeutic efficacy.[8] The obvious advantages of PDX models as screening tools for therapies has really gained traction over the past few years by satisfying an increasing demand for greater genomic diversity amongst humanised models of cancer.[9, 10] However, it is important to point out that many of the cell lines that successfully form PDX *in vivo* are inherently very aggressive tumours and are therefore not reflective of the diversity of cancer phenotypes. PDX development is also laborious, expensive and are not always efficacious. This is a particular problem with estrogen receptor (ER) positive breast cancers which unlike more aggressive subtypes do not readily develop into stable, transferable tumours *in vivo*.[11, 12] Considering that ER positive tumours account for ~75% of all breast cancer diagnosed this is a major failing. Combined with mounting pressure on researchers to adhere to the fundamental principles of humane animal research (3Rs: Replacement, Reduction and Refinement) there has been increasing interest in adapting 3D patient derived models that are not dependent on *in vivo* propagation.

Patient derived organoids

Organoids provide a physiologically relevant alternative to PDX, retaining the 3D architecture of the original tumour whilst avoiding mouse host-driven evolution.[13] The refinement of patient derived organoid (PDO) development and expansion by the research group of Hans Clevers has rapidly propelled the use of PDOs as screening tools for drug sensitivity.[14] Numerous studies have reported the robustness of response and the clinical relevance of the data generated from such screening methods. Indeed there are numerous advantages to organoid expansion of tumour material including reproducibility of response after numerous passages and the maintenance of original genomic and proteomic profiles. Some researchers have cautioned that due to the elaborate culture conditions that there may be some potential interference when using organoids as drug screening tools, in particular the inclusion of cytokines has proven to be most problematic.[14, 15] Additional considerations are that PDO are derived from only one cell type, similar to 2D cultured cells, and they are vulnerable to genetic drift over time. However, one of the biggest disappointments has been that not all tumours will readily form organoids, particularly breast cancers.

3D bio-printing of Patient derived tumour cells

Bio-printing is the spatially controlled positioning of biological materials, living cells and bio-chemicals to produce structures that are biologically functional and mimic the *in vivo* environment. This technology has been utilised in the area of regenerative medicine for a while now and there is increasing interest in using it as a therapeutic screening strategy for cancers. There are a number of different technologies that have been adapted for this purpose namely: ink-jet, extrusion and laser-assisted bio-printing (16). Ink-jet bio-printers are often referred to as 'droplet-on-demand printers' and are by far the most popular and widely available devices for the printing of biological material. A major advantage of this approach is that it is rapid, precise and produces uniform droplets with consistent cell density. Another advantage is that depending on the composition of the bioink used to print the living cells it is also possible to modify the rigidity of the resulting structure so that it can be adapted to most closely replicate the tissue of origin *in vivo*.[17] Newer bioinks also have greatly improved optical properties with very low background fluorescence which allows for more detailed downstream analysis (see figure). Labs including ours are now exploiting this technology for the bio-printing of patient-derived tumour cells

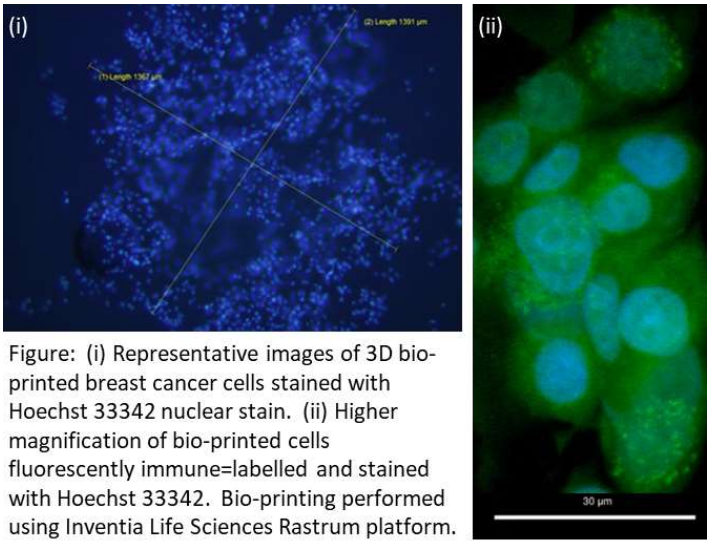
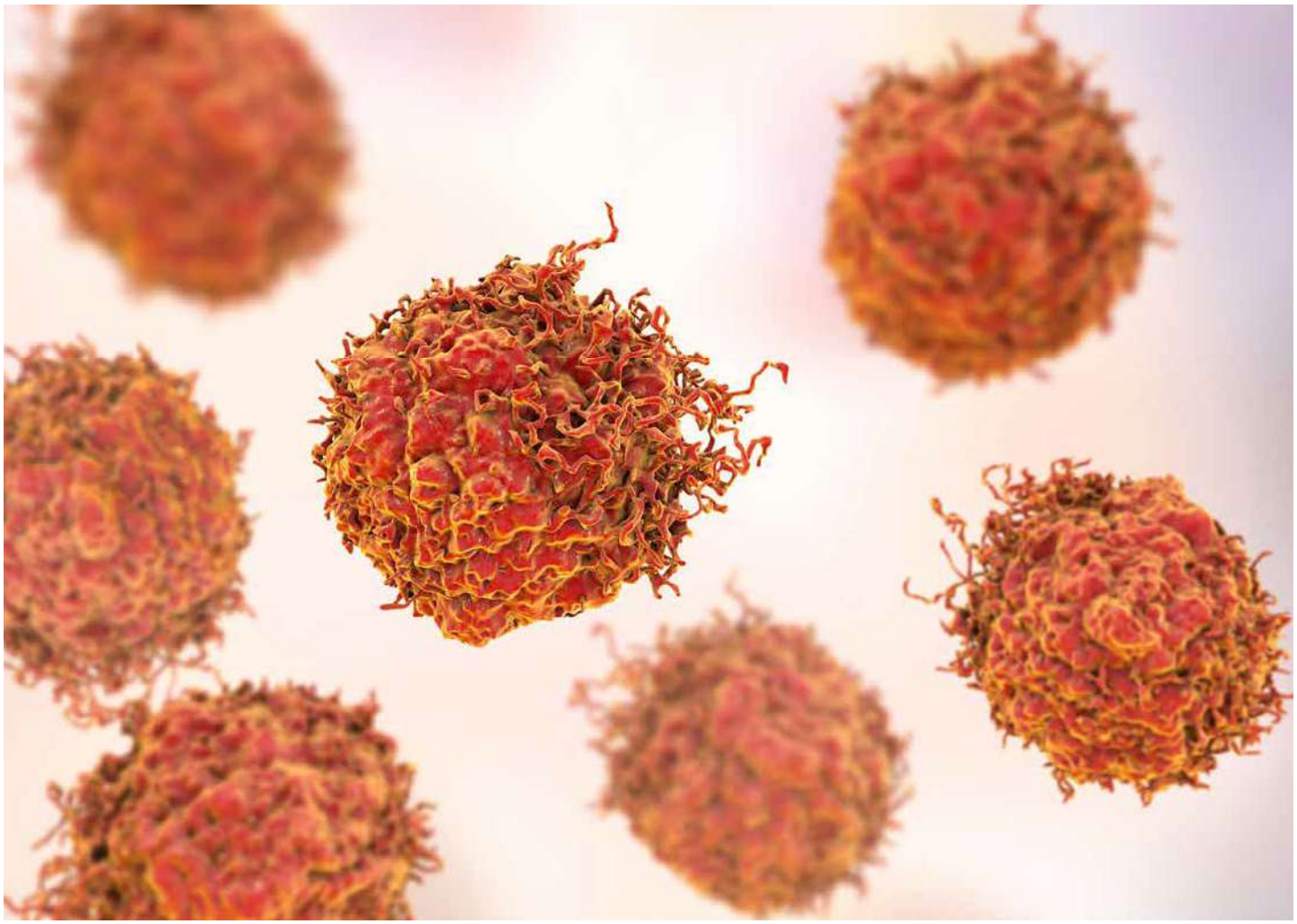


Figure: (i) Representative images of 3D bio-printed breast cancer cells stained with Hoechst 33342 nuclear stain. (ii) Higher magnification of bio-printed cells fluorescently immune=labelled and stained with Hoechst 33342. Bio-printing performed using Inventia Life Sciences Rastrum platform.



with a view to developing this as a high-content screening strategy to evaluate therapeutic response. This is very appealing in the area of hormone receptor positive breast cancers which are notoriously difficult to propagate either as PDX or PDO models. Instead of having to depend on the innate proliferative potential of the tumour, 3D bio-printing will instead allow rapid disaggregation and bio-printing of tumour specimens in a high-content fashion. Once printed, 3D cancer cells can be instantaneously treated with drugs and outputs from the screen collated within days. This is a great advantage over either PDX or PDO which are susceptible to lag periods of cell growth associated with genetic drift. 3D-bio-printing has the capability to enable more efficient and effective screening of therapies targeting tumour metabolism, epigenetics and potentially immune response. The small sample sizes and scale of printing means that ~1 million cells are sufficient to print a 96 well plate opening the possibility of utilising this as a rapid screening tool for metastatic specimens. There are, as always, some downsides to this approach as disaggregation of the primary tumour tissue and cell processing will invariable impact cell viability and cell surface markers in particular. There is also the lack of cellular diversity in the printed structure, however, with advances in the technology, printers are now coming to market with the capacity to print up to eight different cell types.

Using 3D bio-printing to look beyond proliferation/ viability when assessing anti-cancer therapies.

The belief that simply inhibiting cell proliferation would prove to be the Achilles heel of tumour cell immortality has been challenged over the past few decades. The complexity of cancer cell survival encompassing: DNA repair, immune response, metabolism, angiogenic potential and epigenetic changes are now

	Time	Expense	Labour	Physiological relevance
2D culture	days	€	€	∞
PDX	Weeks-months	€€€	€€€	❁
PDO	Weeks-months	€€	€€	❁
3D bio-print	hours	€€	€	❁

TABLE: Schematic with overview contrasting the relative merits of PDX/ PDO and 3D bio-printing approaches in relation to time, expense, labour demands and physiological relevance

accepted to contribute to tumour development and progression. Creating 3D models to enhance the targeting of such nuanced vulnerabilities will require an overhaul of how we profile and screen in advance of clinical testing. Holistic models that layer the proteomic, transcriptomic, epigenetic and endocrine profile of the individual breast tumour will enable unparalleled insight into the drivers of each tumour and deliver a personalised approach to therapeutic screening.

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CPD Module – Multiple Choice Questions:

1. Cell culture of immortalised cell lines are subject to changes in their profile known as

a) Genetic acceleration
b) Genetic drift
c) Genetic stability

2. The original models used to screen anti-cancer agents were mouse____

a) adenoma
b) leukemia
c) lymphoma

3. The development of the ____cell line panel in the 1980's led to the identification of a number of drugs with efficacy against specific cancer

a) NCI-69
b) ABI-19
c) NCI-60

4. Approximately what percentage of compounds fail to progress in Phase II-III clinical trial?

a) 10%
b) 40%
c) 90%

5. Patient derived xenografts (PDX) are singly or serially transplanted tissues/ cells can be derived from

a) Primary tumour
b) Metastasis
c) Both

6. Estrogen receptor (ER) positive breast cancers do not readily develop into stable, transferable tumours in vivo?

True
False

7. There are numerous advantages to organoid expansion of tumour material including reproducibility of response after numerous passages and the maintenance of original genomic and proteomic profiles.

True
False

8. Ink-jet bio-printers are often referred to as _____

a) 'Consistent delivery' printers
b) 'Rapid Process printers'
c) 'Droplet-on-demand printers'

9. Once printed, 3D cancer cells can be instantaneously treated with drugs and outputs from the screen can be collated within:

a) Weeks
b) Months
c) Days

10. The main drawback to 3D bio-printing of primary tumour samples is?

a) Tissue processing
b) Large amounts of material required
c) Slow speed