



Application of Cell-Based Analysis in Evaluation and Quality Control of Traditional Medicines

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Cell-based Assays: History and Definition

- A cell-based assay is:
 - one where the fundamental unit of expression is the cell, either cell populations or single cells
- A cell-based assay normally includes four key elements:
 - A cellular component e.g. a cell line or a primary cell population
 - A target (substrate) molecule that records the cellular response
 - An instrument to conduct and monitor the assay
 - An informatics component to manage and analyse data from the assay
- Cell-based assay has evolved from advances which have:
 - Increasingly identified the molecular mechanisms of disease
 - Stimulated a target-based approach to drug discovery
 - Promoted the adoption of high-throughput screening methodology in drug discovery, rather than empirically-based exploration of pharmacological phenomena (as with traditional medicines)

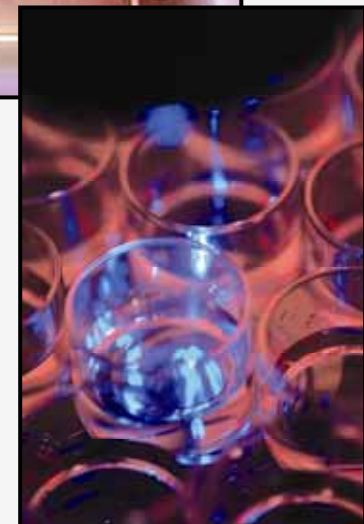
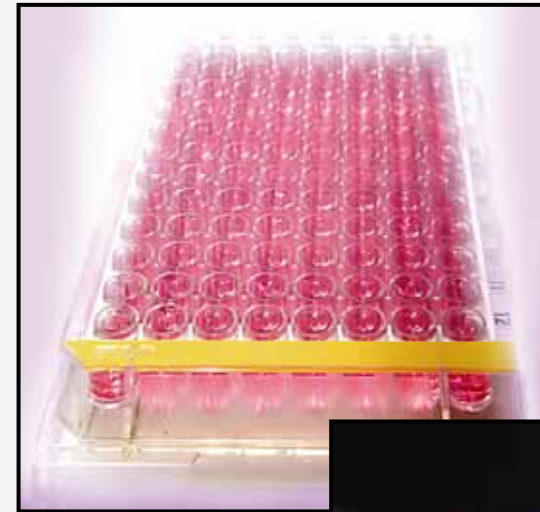
Cell-based Assays: advantages over biochemical assays

- Conformation and activity of the target protein in cellular context
- Assays do not require purification of the target protein
- Can immediately select against compounds / potential drugs that are generally cytotoxic, or that cannot permeate cellular membranes to reach intracellular sites
 - hit/lead compounds identified by cell-based assays have passed important validation steps, saving time and costs in drug development
- Cell-based assays visualise all possible drug-target interactions
 - E.g. activators, target interactions, allosteric modulators



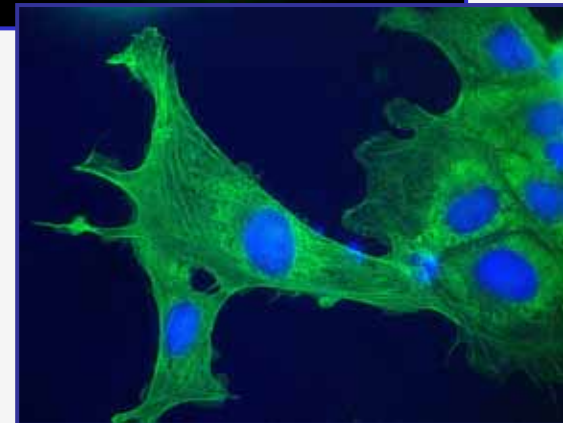
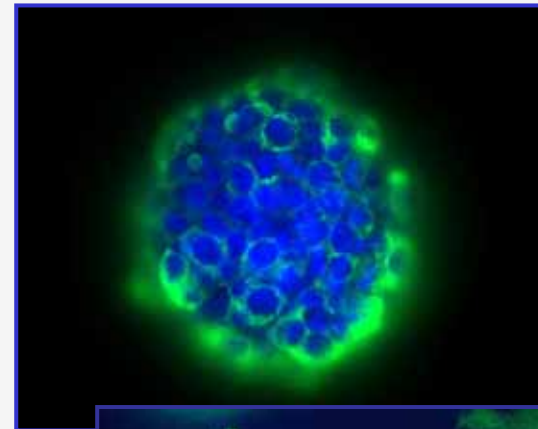
Challenges and Solutions in Assay Development: Cell Culture

- Cell selection
 - Cell lines easy, but unphysiological
 - Primary cells difficult to obtain, but more informative
 - Immortalised differentiated cells, or differentiated stem cells?
- Cell supply, cell selection
 - better (automated) cell growth systems
 - novel treatments to cryo-preserve cells
 - FACS cell sorting
 - better tools for cell immortalization
- Development of robust assays
 - improve dispensing technology
 - incorporate methods for standardizing cell performance
 - include precise handling protocols, minimise need for user intervention
- 3-dimensional cell culture
 - Next generation AvantiCell assays will include 3D cell culture
 - 3D culture enhances cell performance, making results more physiologically relevant



Challenges and Solutions in Assay Development: Assay Readout

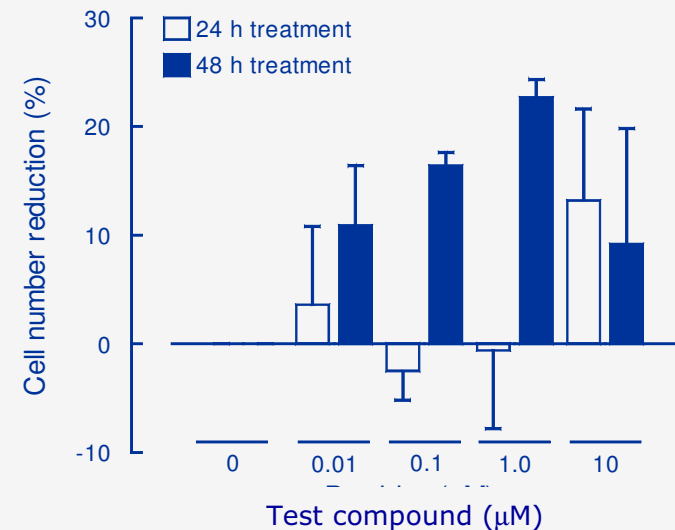
- Assay readout should:
 - deliver selectivity amongst closely-related targets
 - retain assay potency and physiological properties
 - minimise autofluorescence, other background artefacts
 - allow multiplexing of assays (> 1 parameter from a single sample)
- These are likely to be delivered by moving from fluorescence intensity assay to fluorescence lifetime measurement
 - in multiwell plate formats
 - with instrumentation flexibility to enable complex assay formats and multiplexed measurements
 - with bespoke-designed substrates deliverable to intracellular locations



AvantiCell Science: assay example

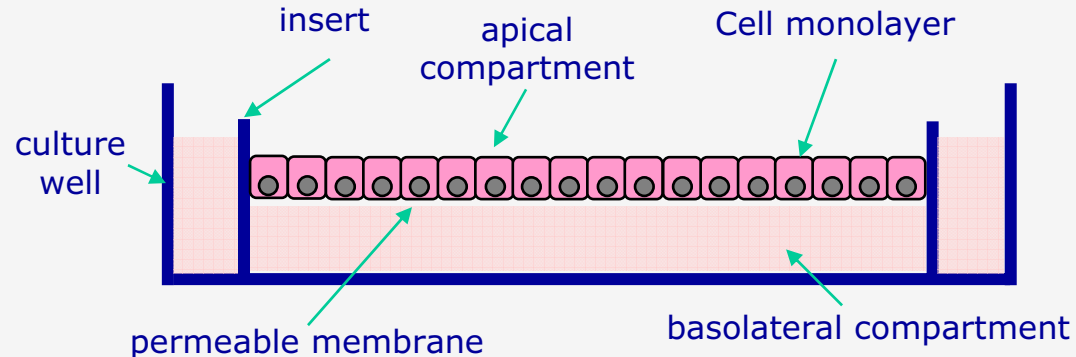
- Customer: academic organisation
- Requirement: testing of potential anti-cancer treatment
- Contract used primary cells and tumour cell line in 96-well formats
- Treatment with candidate therapeutic over 24-48 h cultures
- Fluorescence detection of viable cell number
- Candidate therapeutic reduced viable cell number in cancer cell lines, and was less potent against normal human mammary cells (data not shown).
- Reproducible cell performance and scale of response to test compound across experiments
- Data used by customer for second patent filing; therapeutic now progressing through pre-clinical testing.

Example: reduction in tumour cell number by candidate therapeutic. Data from 6 assays.



AvantiCell example: novel culture system

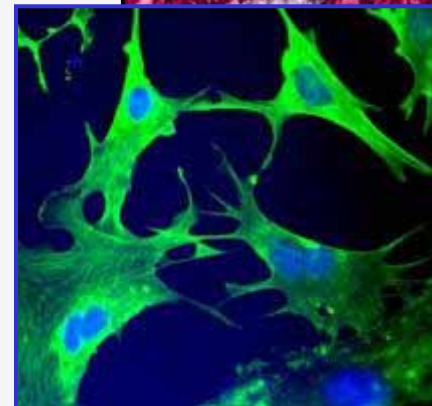
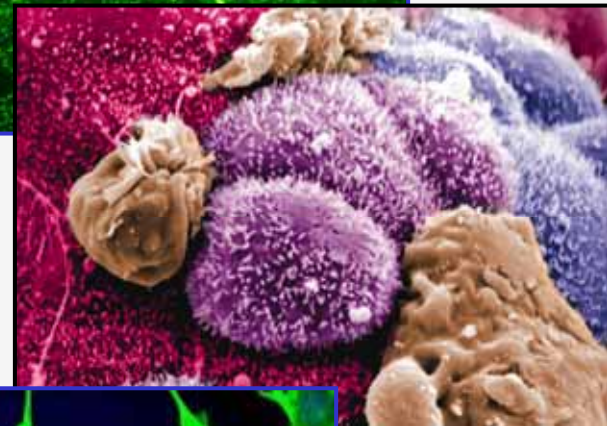
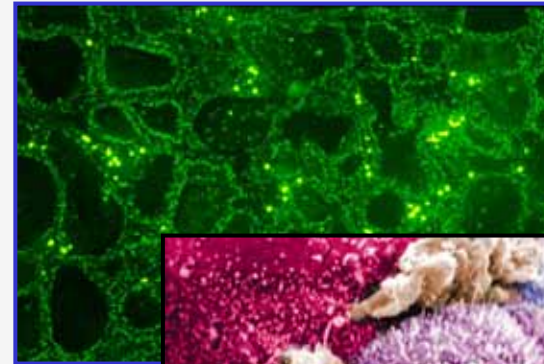
- Client: agri-biotechnology company
- Client requirement: culture system to test in xenobiotics are accumulated in cow's milk and enter the human food chain
- System developed: mammary cells cultured on permeable membrane



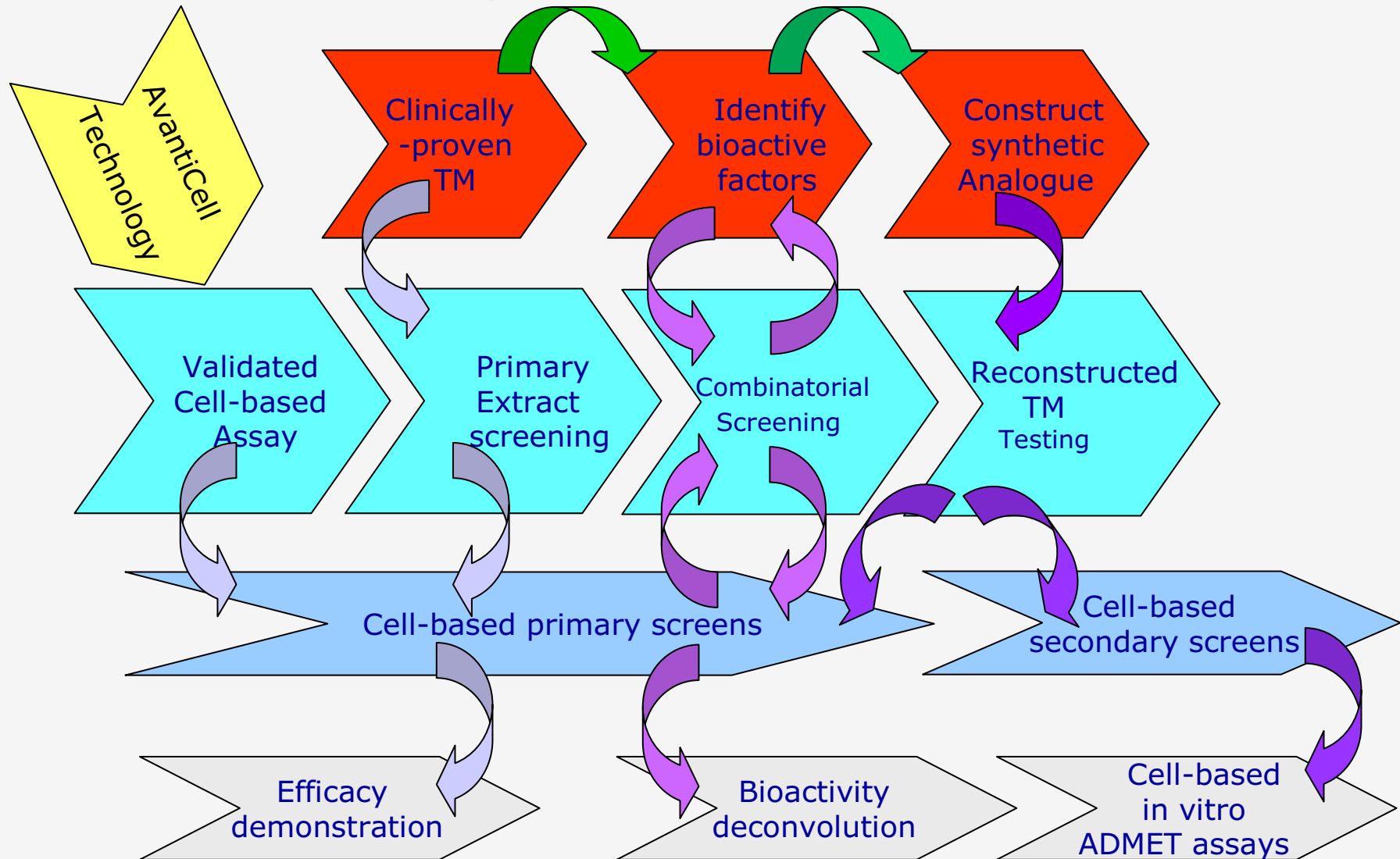
- Cells form polarised cell monolayer, acquire ability to transport molecules vectorially i.e. culture system behaves like tissue *in vivo*, taking in nutrients basolaterally, and secreting milk constituents in an apical direction
- Polarized cell cultures reflect the transport characteristics of test molecules into milk in dairy cow, or in human mother
- System has value in predicting transport of bioactive environmental agents into the human food chain

Cell-based Assays and Traditional Medicines

- Research and Development
 - Demonstration of bioactivity
 - Identification of bioactive constituents
 - Reconstruction of TM from one or more synthetic components
- Quality control of production
 - Cell-based bioactivity measurement
 - QC standard based on chemical finger-printing and cell-based assay bioactivity

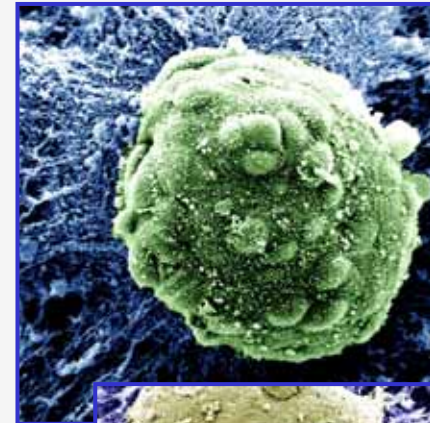


Cell-based Assays: Traditional Medicines



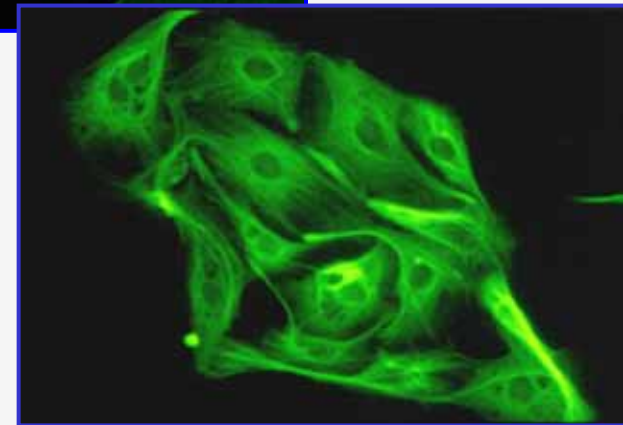
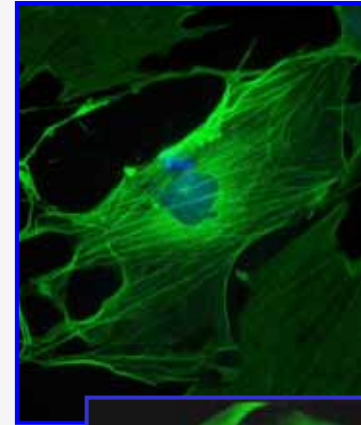
Cell-Based Quality Control of Traditional Medicines

- Adaptation of standard cell-based formats for:
 - Presentation of TM in cell-biocompatible formats
 - Biocompatible solvents and solvent cocktails
 - Stabilization of cells using specialised culture substratum
 - Minimising of auto-fluorescence by TM constituents
 - time-resolved fluorescence
 - fluorescence lifetime
 - Production of multi-target assays containing
 - multiple cell types
 - multiple readouts (multiplexing)
- Cell-based assay kits
 - For customer's use in-house
 - For performance with minimal operator intervention
 - To demonstrate efficacy **and** safety
 - For use alongside chemical fingerprinting (HPLC chromatography etc)



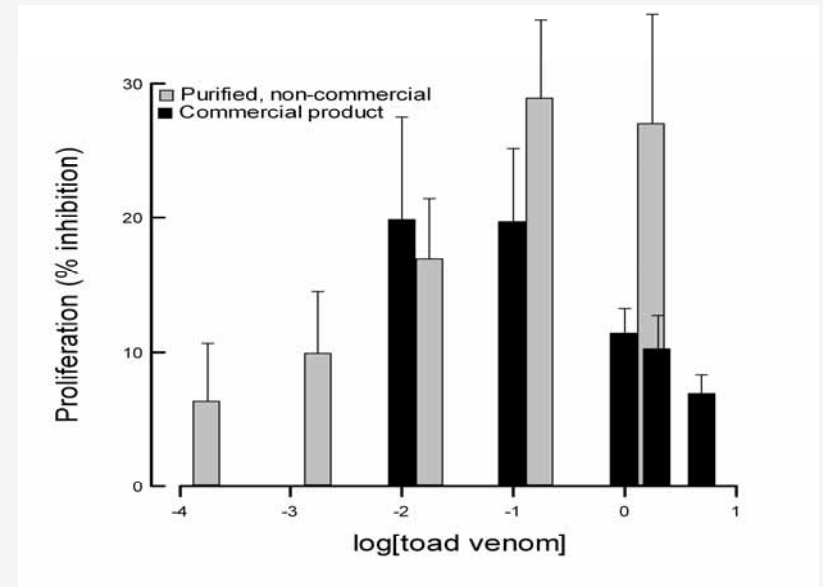
Cell-Based Assay of a Traditional Medicine: A Case Study

- Cell-based assay of toad venom, a known anti-cancer Traditional Chinese Medicine
- Cell-based assays used three indices of cell health: proliferation, cytotoxicity, apoptosis
- Experimental strategy
 - Demonstrate assay's ability to detect the known active ingredients, using preparation of arenabufagins
 - Assess "therapeutic value" i.e. efficacy (anti-proliferation) versus safety (cytotoxicity) and on-target (cancer cell) versus off-target (normal human cell) effect
 - Assess ability to detect bioactivity in clinical preparation, using purified bioactive component as positive control
 - Confirm therapeutic value of clinical product i.e. efficacy versus safety
 - Is toad venom anti-cancer bioactivity only due to inhibition of proliferation, or is apoptosis affected?



Traditional Medicine Case Study: Results

- AvantiCell cell-based assays detect biological activity in a commercial preparation of toad venom TCM, and demonstrate the bioactivity of the major therapeutic component of toad venom, arenobufagin.
- Anti-cancer activity of toad venom TCM and its major bioactive component is measurable as an inhibition of human breast cancer cell proliferation.
- Over the concentration range in which cancer cell proliferation is inhibited, the purified bioactive component, arenobufagin, was not cytotoxic to breast cancer cells, nor was any cytotoxicity detected in cultures of normal human breast epithelial cells.
- Commercial preparations of toad venom TCM similarly showed little or no cytotoxicity against breast cancer or normal breast epithelial cells at concentrations which inhibited cancer cell proliferation.



Inhibition of cell proliferation of human breast cancer cells by commercial preparations of toad venom TCM. Injectable TCM for clinical use was tested over a range of strengths ranging from 2x concentrated (10 ug/ml) to 1:200 (0.01 ug/ml). The inhibitory effect of laboratory-purified component at equivalent concentrations is shown for comparative purposes. Results are the mean \pm SEM.

AvantiCell Science: Cell-Based Assays

- Targeting key events in cancer and other disease states
- Primary cells and cell lines make data “physiologically-relevant”
- Assays bench-marked for use in traditional medicines research and development and in quality control of traditional medicines
- Next generation assays to incorporate 3D cell culture and enhanced fluorescence outputs



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