A 3D model of breast cancer: towards **replacing** the need for animal experiments

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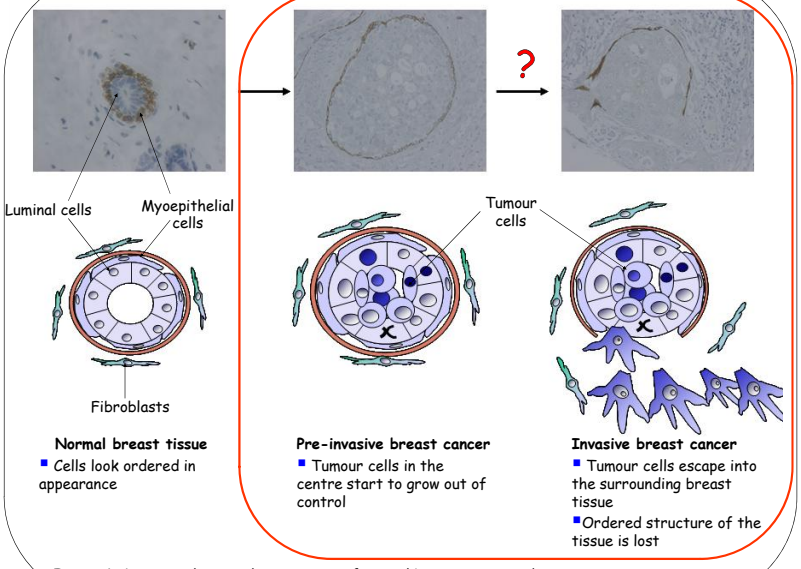
Breast cancer: The problem

- Over 45,000 new cases of breast cancer are diagnosed and more than 12,000 women die of the disease every year.
- Whilst the events leading to breast cancer development are not fully understood, a pre-invasive lesion (ductal carcinoma in situ) is recognised as the main precursor of invasive disease (Figure 1).
- Of these pre-invasive lesions approximately 30% of patients will go on to develop invasive breast cancer which can lead to spread of cancer to other parts of the body (metastasis) which can eventually result in death.
- Understanding how pre-invasive lesions develop into invasive breast cancer is critical as there is no way of knowing which tumours will progress which can lead to unnecessary surgical intervention or unpleasant chemotherapy.

→ To do this we need a good way of modelling breast cancer within the laboratory.



Normal breast tissue
- Cells look ordered in appearance

Pre-invasive breast cancer
- Tumour cells in the centre start to grow out of control

Invasive breast cancer
- Tumour cells escape into the surrounding breast tissue
- Ordered structure of the tissue is lost

Figure 1: Diagram showing the structure of normal breast tissue and progression to invasive cancer

Mouse models of breast cancer:
- Mice are **injected with tumour cells** or mice are **genetically modified** so that they develop cancer.
- Typically these experiments **use over 100 mice** and **experiments can last up to 30 weeks.**
- The tumours look similar to human breast cancer

BUT
- The biology of human and mouse breast tissue is very different
- It is difficult to understand how individual cell types e.g. fibroblasts contribute to the cancer.

Human models of breast cancer:
- Have so far been very simplistic, containing 1 or 2 cell types.
- No human models are available which accurately and reproducibly mimic the biology of human breast cancer progression.

Designing a better human model of breast cancer:
- The model would need to include 3 major cell types involved in breast cancer:
  - Tumour cells.
  - Myoepithelial cells.
  - Fibroblasts.
- Cells would need to be grown in culture conditions which resemble those in the body:
  - Able to grow in 3 dimensions rather than on a plastic Petri dish.

Such a model would be a valuable tool:
- To help us understand how breast cancer progresses.
- To allow us to test new drugs for therapy.
- To potentially identify new targets for future drug development
- As a more biologically and clinically relevant **replacement** to current animal experiments .

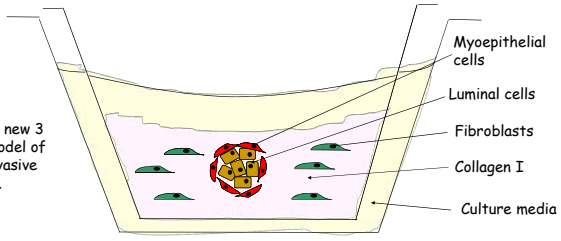


Figure 2: Our new 3 dimensional model of human pre- invasive breast cancer.

Method:
- We have developed a 3 dimensional model which mimics breast cancer and that we are able to grow under well defined laboratory conditions (Figure 2).
- We isolated cells from normal breast tissue or from breast cancer tissue and grew then in a 3 dimensional matrix of collagen.
- By labelling our cells with different colours we were able to identify the different cell types in our model.
- We used the model to investigate whether fibroblasts are able to make pre-invasive lesions become invasive.

Results:
- Our model is the **first of its kind** to grow the 3 major cell types involved in breast cancer: tumour cells, myoepithelial cells and fibroblasts in one system (1).
- By labelling our cells with colours we are able to watch how our model changes over time.
- After 7 days the model looks like human pre-invasive breast cancer (Figure 3a).
  - Blue tumour cells are in the centre
  - Red myoepithelial cells surround the tumour cells
  - Green fibroblasts are outside the tumour cell-myoepithelial unit
- If we include fibroblasts isolated from breast cancer tissue (rather that fibroblasts isolated from non-cancer containing breast) we are able to mimic progression to invasive breast cancer (Figure 3b)
- This shows that the fibroblasts play a very important role in the progression of pre-invasive breast cancer.

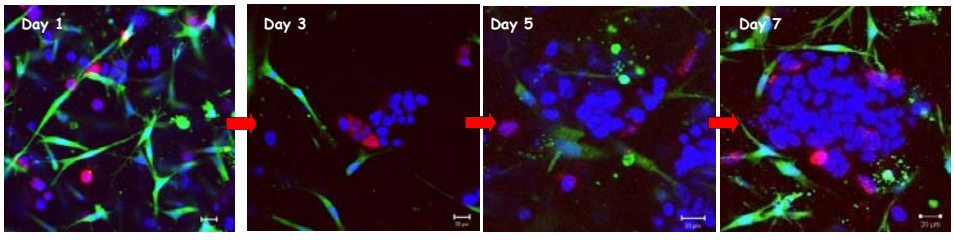


Figure 3a: Growth of the 3d breast model. Blue cells: tumour cells, red cell: myoepithelial cells, green cells: normal fibroblasts. After 7 days we have a model of pre-invasive breast disease

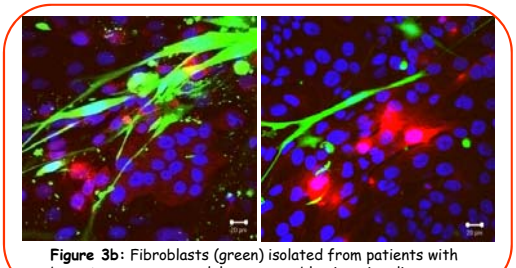


Figure 3b: Fibroblasts (green) isolated from patients with breast cancer, our model now resembles invasive disease

Quantifying our models
- Even though we can see the dramatic effect of tumour fibroblasts on pre-invasive breast cancer we need a way of measuring this.
- Using a microscope we can count the number of individual structures formed (Figure 4a).
  - With normal fibroblasts we see many distinct ordered structures.
  - With tumour fibroblasts the ordered structures are lost.
- This gives us a measure of breast cancer progression which we can plot on a graph (Figure 4b).
- This becomes very important if we want to study how drugs will affect breast cancer progression.
- It allows us to measure of how effective drugs are at preventing tumour invasion which is the critical step in treating breast cancer.

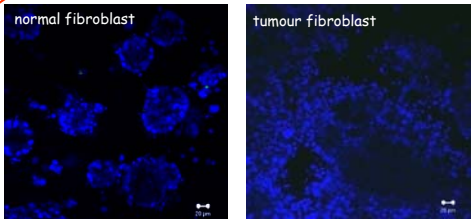


Figure 4a: low power magnification of the 3d breast cancer model.

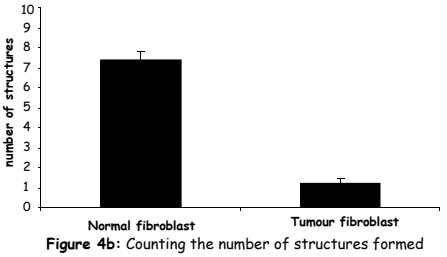


Figure 4b: Counting the number of structures formed

Future plans and conclusions:
- Currently we are manipulating our 3D model to develop a series of models which will represent the many different types of breast cancer. We will use these models to further investigate how fibroblasts are able to promote breast cancer progression.
- Our models are **advantageous over animal models** as they **more closely represent human disease** and unlike animal experiments allow us to mimic the complex cellular interactions occurring in breast cancer, an essential step in understanding breast cancer behaviour.
- The models provide a **replacement** for animal experiments by giving us a **human system** to study breast cancer biology and drug treatments.

Dr Hadwen Trust
Replacing Animals in Medical Research

(1) Novel multicellular organotypic models of normal and malignant breast: tools for dissecting the role of the microenvironment in breast cancer progression. DL Holliday, KT Brouilette, A Markert, LA Gordon, J.Louise Jones. (2009) Breast Cancer Research 11:R3

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