Better Models for Better Breast Cancer Research:

Could 3D models of non-invasive breast cancer reveal the key to invasive progression?

Sarah McLucas[1, 2], Barbara Guild[1], Gemma Wilson[1, 2], Nirmala Pathmanathan[2,3] and Dinny Graham[1, 2, 3]

[1] Centre for Cancer Research, The Westmead Institute for Medical Research, Westmead
[2] Faculty of Medicine and Health, University of Sydney, Sydney
[3] Breast Cancer Institute, Westmead Hospital, Westmead

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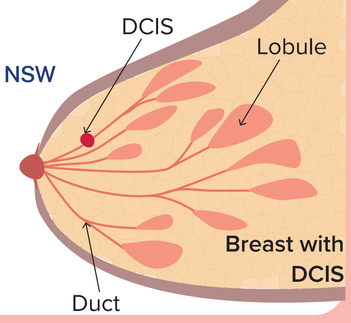
Sydney Cancer Partners
Collaboration, Translation, Impact



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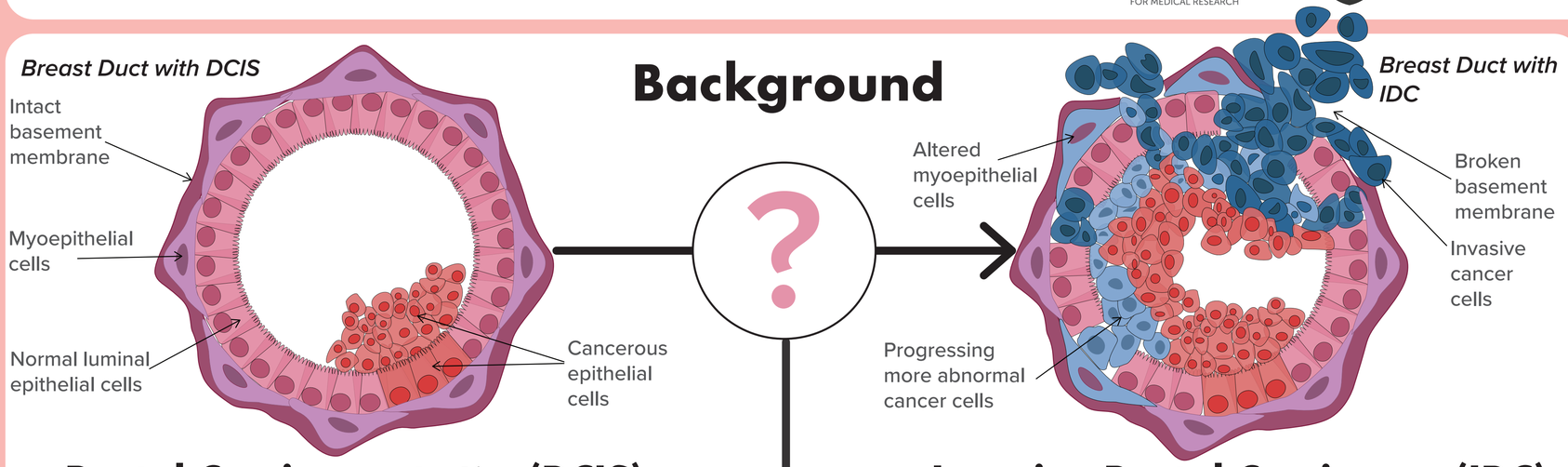


Ductal Carcinoma *In Situ* (DCIS)

- Preinvasive breast cancer.
- Confined within the mammary ducts.

As few as 12% of lesions will progress if left untreated. [1]

Invasive Ductal Carcinoma (IDC)

- Cancer cells escape duct to invade the stroma
- No reliable method to predict invasion.
- All patients undergo surgery and radiotherapy
  ⇒ unnecessary treatment in many cases.

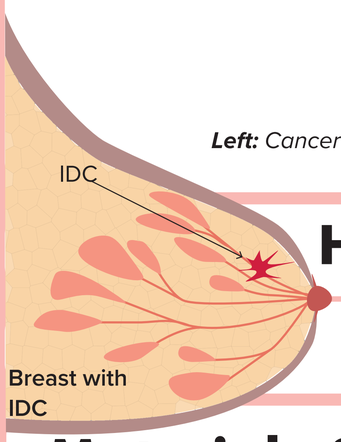


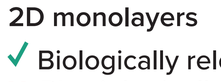
Figure 1: Progression of DCIS to IDC is unpredictable
Left: *Cancer cells (red) confined by normal myoepithelium.* ***Right:*** *Progressively altered cancer cells (shown by colour change light to dark blue) cross the myoepithelium and basement membrane to invade surrounding stroma.*

Significance

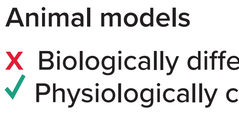
Breast cancer is the most diagnosed cancer globally[2] **20% of breast cancer detected by screening is DCIS.**[3]

A **small fraction of DCIS progresses** to IDC, **BUT all patients are treated** as if they will develop invasive disease.

Current research paradigms have not revealed the key to the invasive transition.

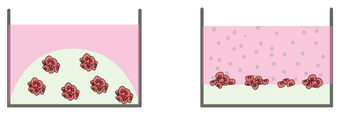


2D monolayers
✓ Biologically relevant
X Physiologically simple



Animal models
X Biologically different
✓ Physiologically complex

3D models overcome current limitations representing a promising new paradigm for studying cancer.



✓ Biologically relevant
✓ Physiologically complex

Hypothesis

Novel 3D culture approaches **better** model DCIS and its invasive transition to IDC.

Aim

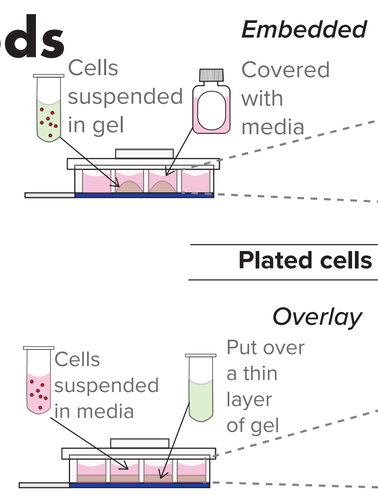
To compare and optimise 3D culture conditions for **better** modelling DCIS in vitro using continuous and primary cell lines.

Materials & Methods

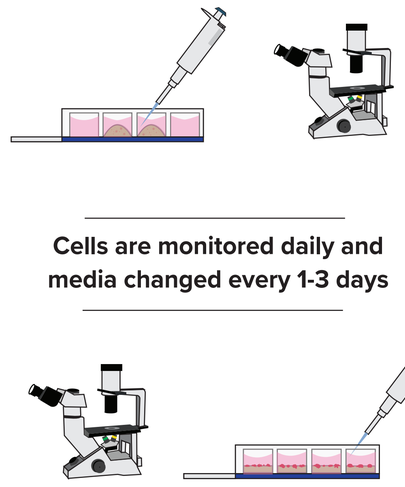
We adapted two normal mammary culture models[4] using the DCIS.com breast cancer cell line

Parameters tested

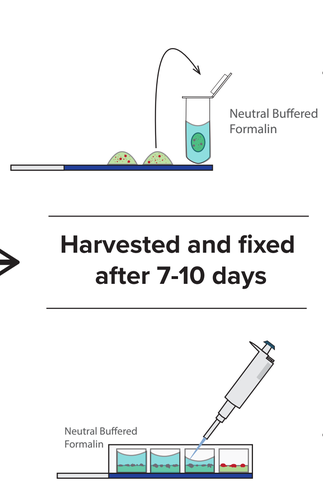
- ☐ Seeding density:
  5,000- 50,000 cells/well
- ☐ Matrix gel composition
  - Matrigel
  - Collagen I
- ☐ Presence or absence patient-derived fibroblasts



Cells are monitored daily and media changed every 1-3 days



Harvested and fixed after 7-10 days



Paraffin embedding, sectioning and staining

Immunofluorescent staining on culture slide

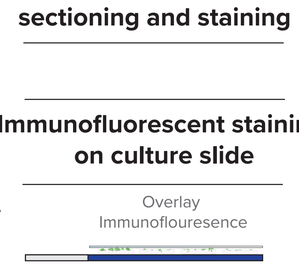
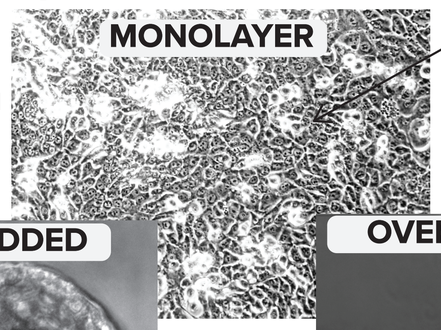


Figure 2: Methodology overview for overlay and embedded cultures

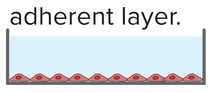
Results

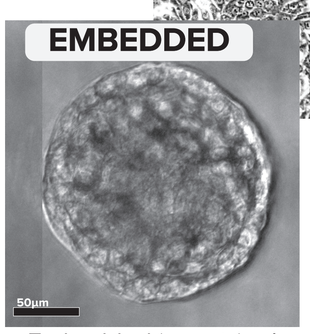
Figure 4: Bright-field microscope image of flask of DCIS.com cells. Differential interference contrast (DIC) image of overlay culture. DIC image and H&E section of embedded cultures.

DCIS.com is an immortalised cell line used to model DCIS *in vitro*.



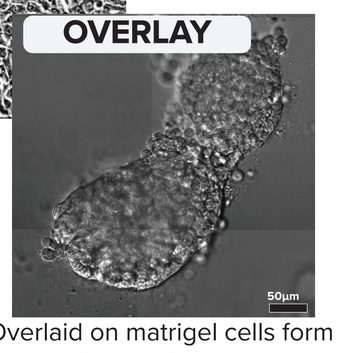
Plated in monolayer cells form a flat adherent layer.





Matrigel rich in basement membrane components: laminin, collagen IV & fibronectin

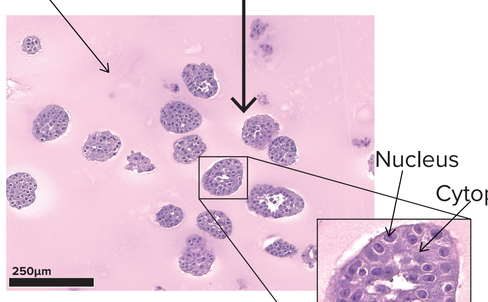
Embedded in matrigel cells form discrete regular spheres.



Overlaid on matrigel cells form large disorganised clusters.

↓

Limited staining options as cultures are fragile, easily disturbed and cannot be embedded.



Hematoxylin- and eosin-stained (H&E) section shows discrete spheres of cells that resemble patient DCIS with the same staining.

Breast duct with low-grade DCIS

Stroma: rich in collagen I

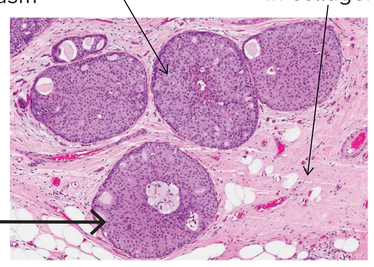


Figure 5: H&E stained section of low grade DCIS from Hanna et al (2019)[5]

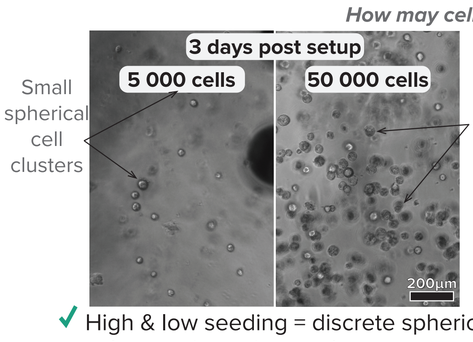
Parameters tested:

Embedded Cultures

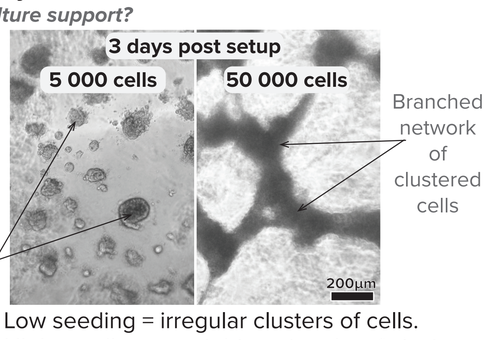
Overlay Cultures

Seeding Density:

How may cells can the culture support?



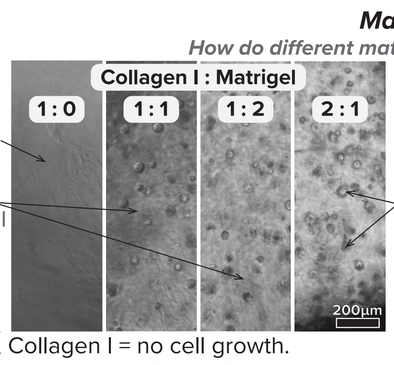
✓ High & low seeding = discrete spherical clusters throughout culture.



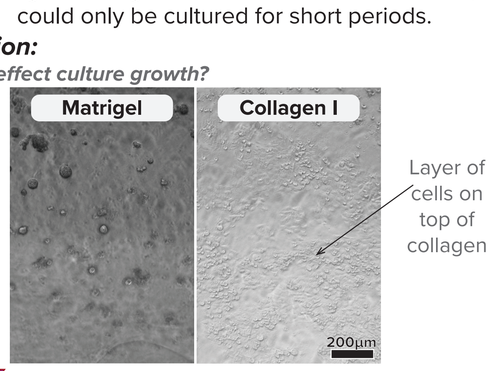
✓ Low seeding = irregular clusters of cells.
X High seeding = unstable networks of clusters, could only be cultured for short periods.

Matrix Variation:

How do different matrix proteins effect culture growth?



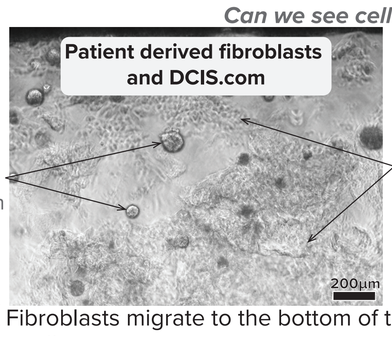
X Collagen I = no cell growth.
✓ Mixtures of collagen & matrigel = clusters.
? High concentration of collagen = more irregularly shaped.



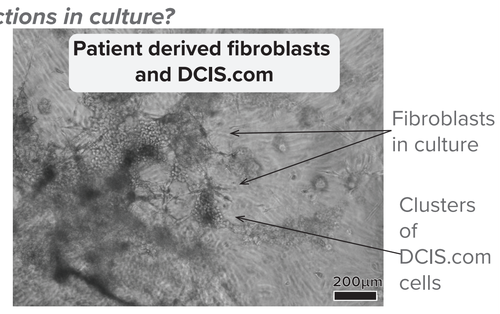
X Collagen I = cells form layer on top of collagen similar to monolayer cultures.

Co-culture:

Can we see cell-cell interactions in culture?



X Fibroblasts migrate to the bottom of the well and adhere.



✓ Fibroblasts remain in culture, resemble *in vivo* morphology, and show cellular interactions.

Figure 6: Bright-field microscope images comparing 3D Culture conditions.

Conclusion

Embedded cultures closely resemble *in vivo* lesions (figure 5) and have significant benefits over the overlay culture system:

Embedded culture system:

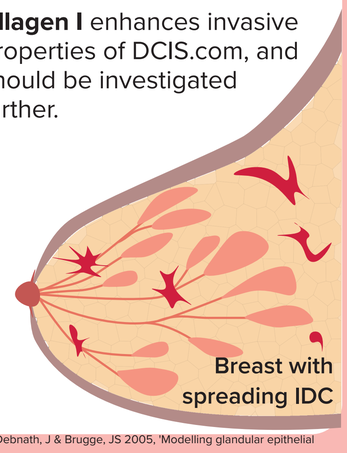
- ✓ is more robust.
- ✓ supports higher seeding densities & has greater cell yield.
- ✓ has more analysis options as it can be paraffin embedded.
- X does not support fibroblasts.

Overlay culture system :

- X is fragile & clusters are easily disturbed.
- X higher seeding densities are not supported.
- X cannot be paraffin embedded.
- ✓ is better at supporting co-cuture with fibroblasts.

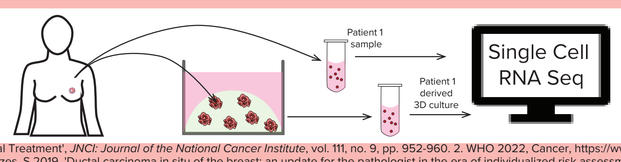
This optimisation has also implicated a role for collagen I:

- ? **Collagen I** enhances invasive properties of DCIS.com, and should be investigated further.



Next Steps

Optimised protocols will be used on patient-derived normal breast tissue and DCIS samples.



Single cell RNA sequencing will be used to see how well 3D culture maintains cell populations and phenotypes seen *in vivo*

1. Ryser, MD, Weaver, DL, Zhao, F, Worni, M, Grimm, LJ, Gulati, R, Etzioni, R, Hyslop, T, Lee, SJ & Hwang, ES 2019, 'Cancer Outcomes in DCIS Patients Without Locoregional Treatment', *JNCI: Journal of the National Cancer Institute*, vol. 111, no. 9, pp. 952-960. 2. WHO 2022, Cancer, https://www.who.int/news-room/fact-sheets/detail/cancer 3. AIHW 2021, 'BreastScreen Australia monitoring report 2021', Canberra, 4. Debnath, J & Brugge, JS 2005, 'Modelling glandular epithelial cancers in three-dimensional cultures', *Nature Reviews Cancer*, vol. 5, no. 9, pp. 675-88. 5. Hanna, WM, Parra-Herran, C, Lu, F-I, Slodkowska, E, Rakovitch, E & Nofech-Mozes, S 2019, 'Ductal carcinoma in situ of the breast: an update for the pathologist in the era of individualized risk assessment and tailored therapies', *Modern pathology*, vol. 32, no. 7, pp. 896-915.