SMALL MOLECULE THERAPIES TO RESCUE GAP JUNCTIONAL INTRACELLULAR COMMUNICATION AND OVERCOME STIFFNESS-INDUCED CHEMORESISTANCE

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Introduction

Breast Cancer

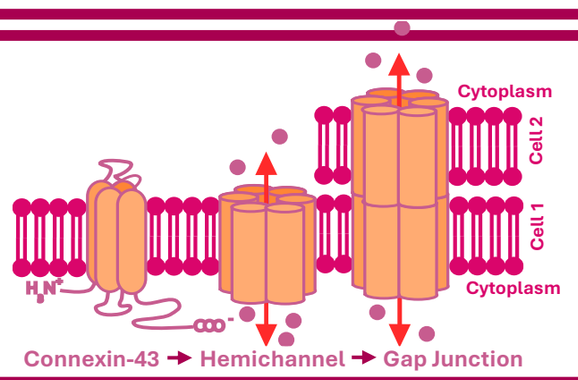
- **Breast cancer (BC)** is the leading cause of cancer death in women worldwide [1].
- Women with **dense breasts** have a higher BC risk
- Tumours promote the development of a **stiff and fibrous** extracellular matrix, which alters cell behaviour and potentially impacts chemosensitivity.

Objectives

1. Determine how stiffness alters breast cancer growth and response to chemotherapy.
2. Identify how GJ activity changes in BC cell lines
3. Assessing gap junctions as potential targets to promote chemotherapy penetration and efficacy in stiffness spheroids.

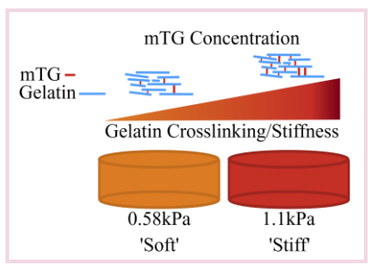
Gap Junctions

- **Gap junctions** (GJs) allow the passage of small molecules between neighbouring cells to coordinate cell growth and behaviour.
- Constructed of protein subunits known as connexins.
- GJs, particularly Connexin-43 (Cx43), are dysregulated in breast cancer and their impact upon tumour development isn't fully understood.



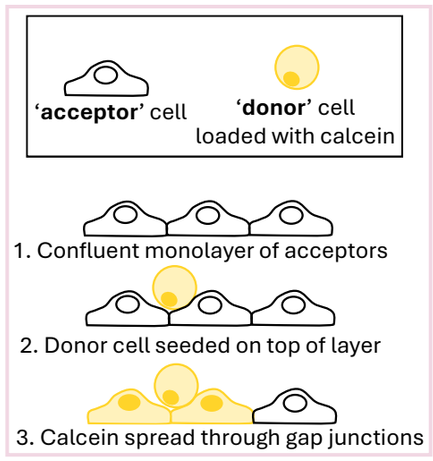
Methods

3D Hydrogel Models



Gelatin hydrogels were crosslinked using microbial transglutaminase (mTG) to alter gel stiffness. Two stiffnesses were selected, a 0.58kPa 'soft' and a 1.1kPa 'stiff' hydrogel. Single T47D cells were seeded in the hydrogels and cultured for 12 days. The hydrogels were treated with 10μM doxorubicin for 48 hours. Imaged using brightfield and confocal microscopy. Spheroid size and volume was calculated using ImageJ.

Cx43 and GJ analysis



Cx43 expression was assessed using immunofluorescence on low passage (LP) and high passage (HP) T47D cells, P1-10 and P11-20 respectively.

Calcein assay: Single T47D cells loaded with calcein were seeded on top of a monolayer of acceptor cells. The spread of calcein was recorded every 15 mins.

Results and Discussions

Mechanically-Induced Chemoresistance

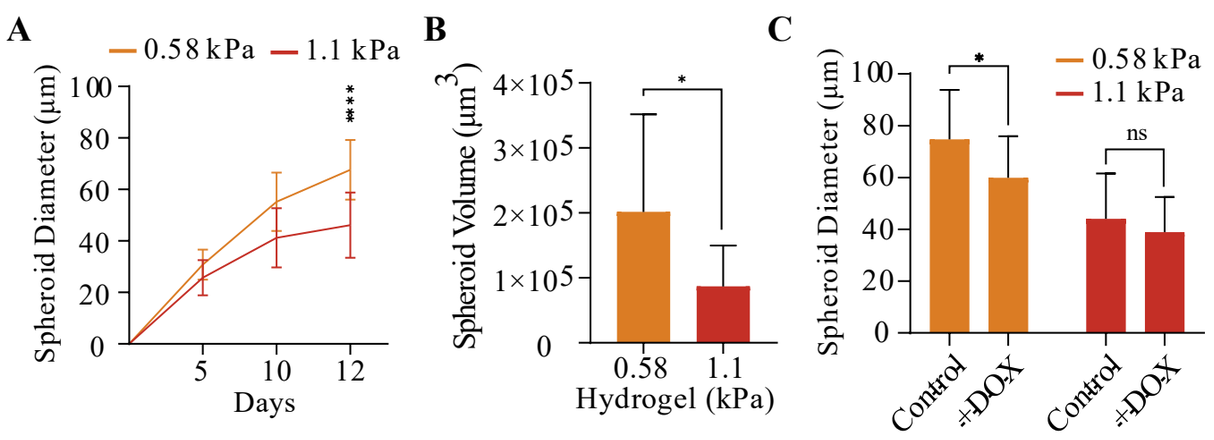
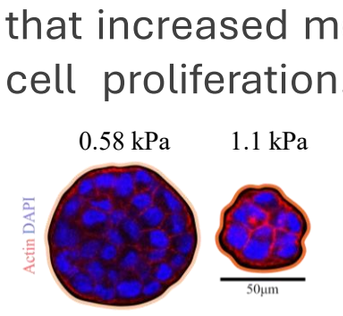


Figure 1. Average spheroid size. A) Change in diameter over culture period. B) Confocal analysis of spheroid volume. C) Following 10uM DOX treatment.

Spheroids in soft hydrogels were significantly larger than the spheroids in the stiff hydrogels (**Figure 1A-B**), demonstrating that increased mechanical loading upon the tumour reduces cell proliferation. Following treatment with chemotherapy, the stiff spheroids did not significantly change in size compared to the soft spheroids (**Figure 1C**), implying a form of mechanically-induced chemoresistance.



Rescuing Gap Junctions

- T47D cells lose Cx43 expression and GJ activity as cells progress through passages (**Figure 2A-C**).
- CX43 is highly localised in the cytoplasm.
- Cx43 expression and localisation to the membrane can be rescued in HP cells through the use of small molecule therapies - Resveratrol (RES), Dipyridamole (DIP), and Retinoic Acid (ATRA). The therapies target different aspects of the GJ assembly pathway (**Figure 2D**).

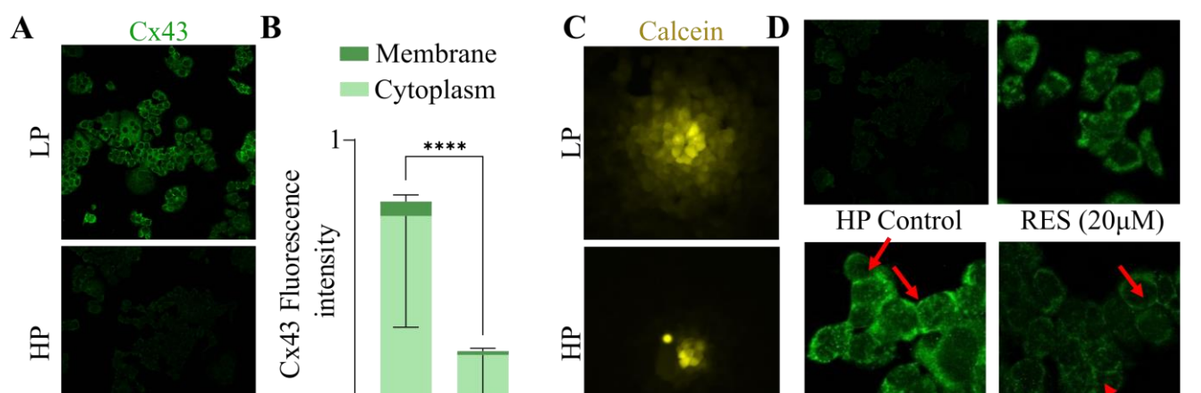


Figure 2. A) Cx43 immunofluorescence (IF). B) Cx43 IF localisation. C) Calcein assay. D) Cx43 IF following 24 hour GJ therapy treatment. Arrows = cell-cell plaques

Combinational Therapy to Promote Chemosensitivity

Samples were pre-treated with GJ therapies before exposure to DOX. In 2D, combination treatment didn't alter viability compared to DOX treatment alone (**Figure 3A**). These results are expected as the cells are all exposed to media, and promoting GJs will not influence chemotherapy penetration.

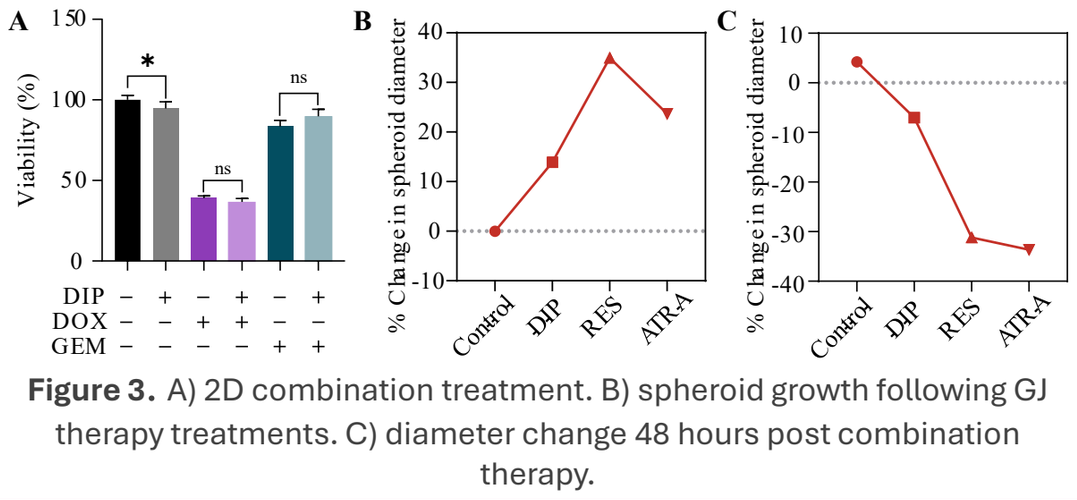


Figure 3. A) 2D combination treatment. B) spheroid growth following GJ therapy treatments. C) diameter change 48 hours post combination therapy.

However in 3D spheroids, the GJ treatments initially promoted spheroid growth (**Figure 2B**) and when followed by DOX treatment, resulted in a decrease in spheroid size which was not observed in DOX treatment alone (**Figure 2C**).

Conclusions

- High mechanical stress induces the development of smaller, more compact spheroids.
- Increased cell density, lower proliferation, and a possible loss in GJ expression interferes with chemotherapy diffusion and sensitivity.
- GJ assembly was rescued through treatment with small molecule therapies.
- These GJ therapies show promise to promote chemosensitivity in stiff tumours.

Acknowledgements

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