Development of a preclinical TNBC tumour model and investigation of a novel targeted therapy

Farhana Mollah[1], Hamidreza Aboulkheyr Es[2], Majid Warkiani[2], Pegah Varamini[1,3]

[1]Sydney Pharmacy School, University of Sydney, NSW, Australia; [2]School of Biomedical Engineering, University of Technology, NSW, Australia; [3]The University of Sydney Nano Institute, NSW, Australia; Sydney

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Introduction

- Breast cancer is the most common cancer in women around the world.
- 15-20% of all breast cancers are Triple Negative Breast Cancer (TNBC).
- TNBC does not have common markers such as ***Progesterone***, ***Estrogen*** and **HER2 protein**.
- There are limited treatment options with predominant nonspecific chemotherapy to which patients develop resistance soon after the first treatment cycle causing TNBC to have the highest risk of relapse among all subtypes and become metastatic (12% 5-year survival)[1].
- Breast Cancer Associated Fibroblast (BCAFs) is a component of the tumour microenvironment, which increases resistance to therapy.
- TNBC is considered an "**unmet medical challenge**" with limited effective therapeutic options.
- In order to develop new effective treatments for TNBC, it is crucial to establish clinically-relevant models of TNBC that incorporate the presence and impact of BCAFs in developing new targeted therapies.
- Since 3D model mimic the function, and architecture of *in vivo* cells, it is necessary to use 3D TNBC models [2]. Additionally, 3D models enables the evaluation of the tumour microenvironment components and their cell-to-cell interaction through coculture.
- **Aims:** i) to develop 3D model of TNBC and coculture (TNBC/BCAFs) ii) evaluate the efficacy of novel anticancer agents using 3D models

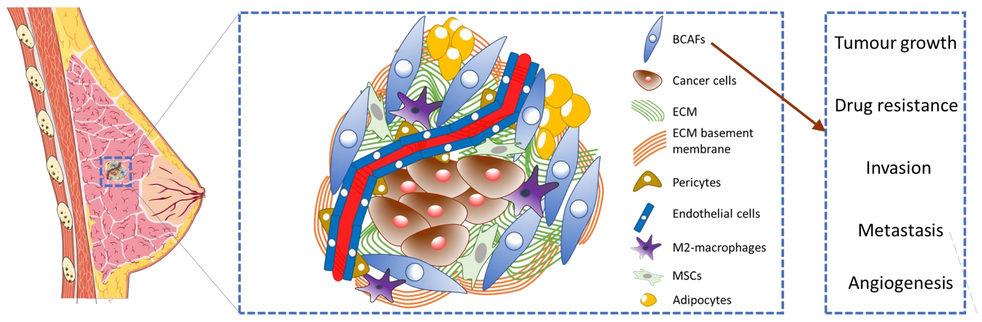


Figure 1. BCAFs in the tumour microenvironment lead to tumour progression through a range of different pathways, including drug resistance and metastasis. **Adapted from:** Mollah, F.; Varamini, P. Overcoming Therapy Resistance and Relapse in TNBC: Emerging Technologies to Target Breast Cancer-Associated Fibroblasts. Biomedicines 2021, 9, 1921.

Results & Discussion (Aim 1)

Development of TNBC and coculture spheroids

- To develop TNBC spheroids, MDAMB231 cells were utilised whereas coculture established from MDAMB231 and human dermal fibroblasts (HDF).

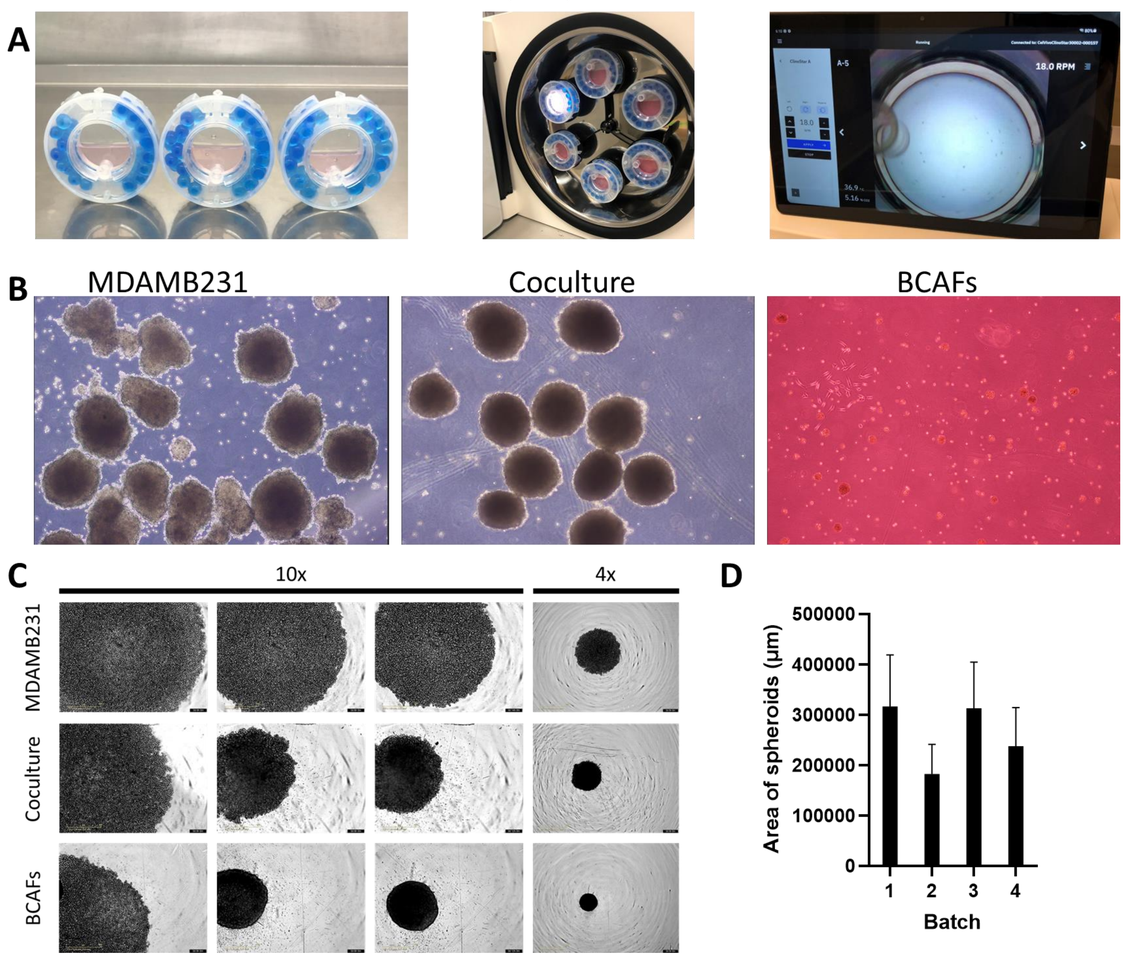


Figure 3. A) Spheroids were developed in ClinoStar™ 3D Bioreactor using reactors **B)** 4x images of TNBC (MBAMB231), Coculture (MDAMB231 & HDF) and BCAFs (transformed HDF) spheroids in reactor **C)** MDAMB231, Coculture and BCAF spheroids development and morphology in 96 well plate using 10x and 4x magnification **D)** Size of seven-day old spheroids (representative MBAMB231) from different batches (n=4).

Figure 4. DAPI, LHRH receptor and αSMA expression levels in MDAMB231, Coculture and BCAF spheroids.

TNBC, Coculture and BCAF spheroids were developed successfully using the method (Fig. 3A). The three types of spheroids are morphologically different (Fig. 3B and 3C). The sizes of the spheroids were varied in different batches (Fig. 3D). The three types of spheroids had similar expression of LHRH receptor and different αSMA expressions (Fig. 4).

Discussion

Since LHRH receptors are overexpressed in TNBC cells, MDAMB231 spheroids are successfully developed. BCAFs express αSMA, however multiple markers are required to characterise them [1]. Additional characterisation is necessary to validate these findings.

Methods

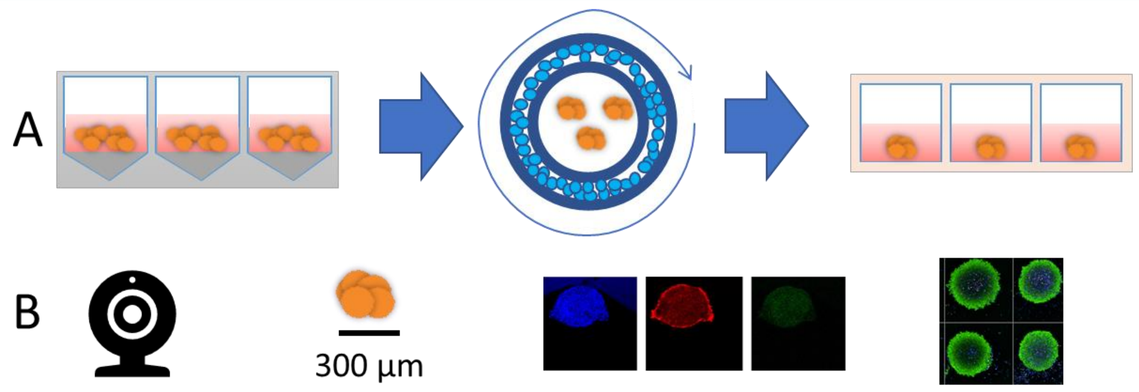
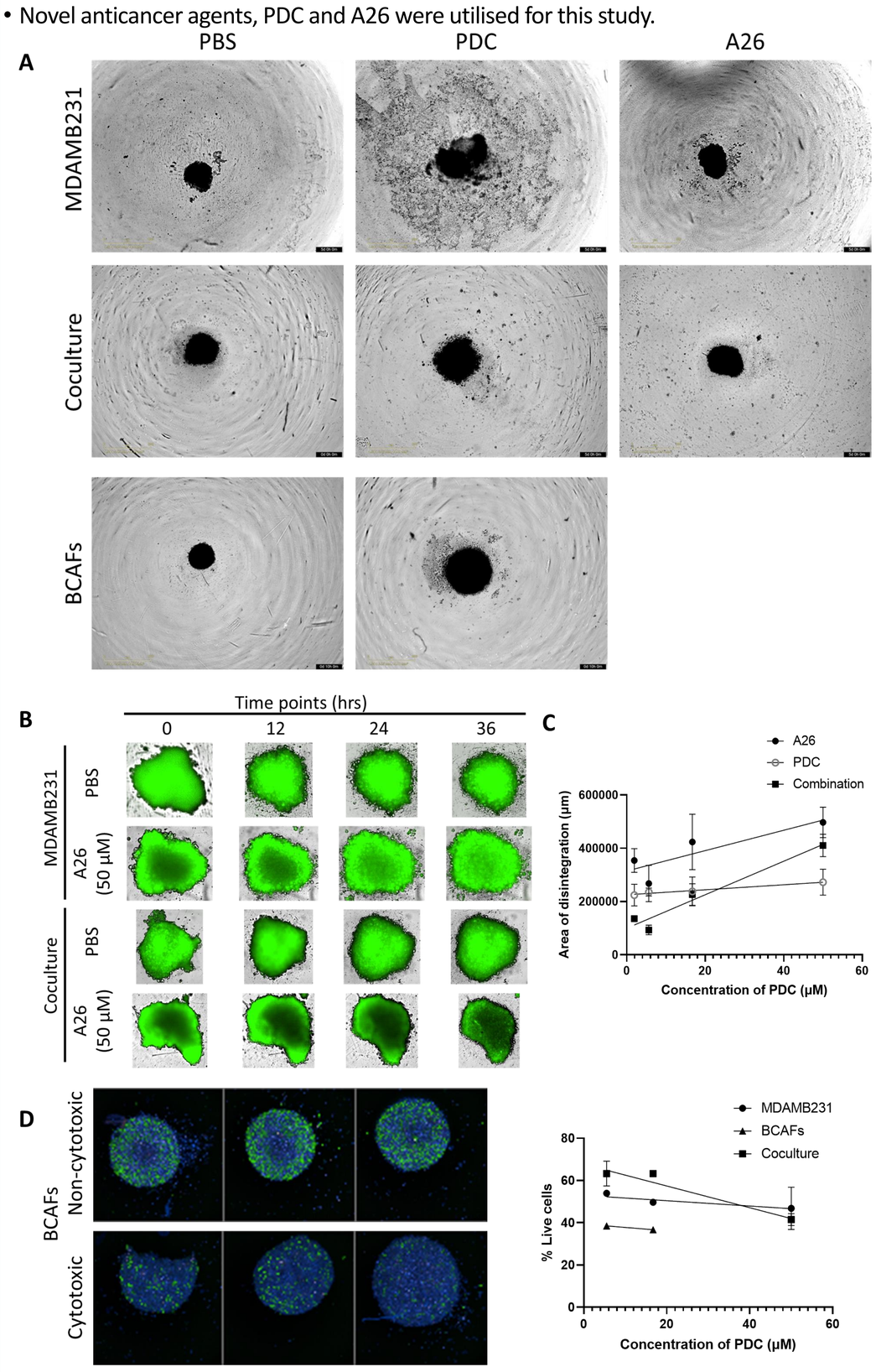


Figure 2. Method of spheroid development and characterisation. **A)** 2D cells were seeded onto agar coated V-shaped 96 well plate, transferred to reactors for ClinoStar™ 3D Bioreactor and finally transferred to ultra low attachment 96 well plate for treatment **B)** The spheroids were characterised by bright field image, area measurements, immunostaining and live and dead assay.

Results (Aim 2)

Evaluation of novel anticancer agents using the 3D models of TNBC and coculture with BCAFs

- Novel anticancer agents, PDC and A26 were utilised for this study.



Incucyte Imaging
Green fluorescence/Bright field microscopy
Phenix microscopy
Live and dead assay

Figure 5. A) Disintegration of MDAMB231, Coculture and BCAF spheroids compared to PBS control following five-day treatment with PDC or A26 **B)** Penetration of fluorescent A26 in TNBC and coculture spheroids compared to the PBS control **C)** Area of disintegration of spheroids (representative MDAMB231) increases as the concentration of PDC and A26 increases **(D)** Live and dead assay images of spheroids (representative BCAFs) treated with cytotoxic and non-cytotoxic agents and quantification of percentage of live cells following PDC treatment on MDAMB231, Coculture and BCAF spheroids

Conclusion and Future directions

TNBC (MDAMB231), BCAFs and Coculture spheroids are unique morphologically and through varying levels of expression of αSMA. Further characterisation will be conducted using more TNBC and BCAF specific markers.

A potential synergistic effect on cell death occurred after combining PDC and A26. Additional live and dead assays, cell viability (quantitative measure) and invasion assays will be conducted to further validate these findings.

References

[1] Mollah, F.; Varamini, P. *Overcoming Therapy Resistance and Relapse in TNBC: Emerging Technologies to Target Breast Cancer-Associated Fibroblasts.* Biomedicines 2021, 9, 1921.

[2] Van Zundert I, Fortuni B, Rocha S. *From 2D to 3D Cancer Cell Models-The Enigmas of Drug Delivery Research.* Nanomaterials (Basel). 2020 Nov 11;10(11):2236.