Studentship Report: The Impact of Collagen III on Breast Cancer Cell Morphology Cultured in Functionalized Hydrogels

Aims

The primary aim of my project was to look at the impact of collagen III on breast cancer cell morphology in cells cultured in functionalized hydrogels, contributing to ongoing research into breast cancer cell progression. This work was part of a wider collaboration between the Ashworth and Merry research groups, utilizing PeptiMatrix technology to create 3D biomaterial models that mimic the extracellular matrix (ECM) of diseased tissues. By examining the interaction between breast cancer cells and their surrounding microenvironment, the project sought to provide insight into the role of collagen III in cancer progression.

I aimed to explore how 3D biomaterial models could improve our understanding of the complex relationship between cancer cells and their ECM. Additionally, this project was designed to lay the groundwork for further research, with a Master's student taking over once I finished, setting up the development of a more refined breast cancer culture system to be taken forward by future researchers.

Summary of work

Firstly, to cultivate breast cancer cells for my project, I began by receiving training in cell culture, which was completed inside a microbiological safety cabinet. We used MCF-7 cells; an established breast cancer cell line commonly used in research. I learned how to culture cells, using techniques such as media changes and cell passaging to develop experiment-ready cell cultures. After a few passages, the cells were inserted into hydrogels I had made up using PeptiMatix techniques. I incorporated collagen I and III into some of the gels to observe if it affected cell morphology (**Fig 1.**). After the cultures were given some time to grow in the gels, I completed a live/dead viability assay (**Fig 2.**) to determine if the cells were viable to experiment with. To enhance microscope imaging, I then fixed the cells using paraformaldehyde to suspend them in place, then embedded them into an agarose gel which was subsequently sectioned into thin slices using a vibratome. Using stains to detect the presence of collagen I and III, I took images of the gels using a confocal microscope (**Fig 3.**).

Outside of the lab, I attended biweekly research group meetings, where researchers at the university presented their work and noteworthy findings. I had the opportunity to contribute to this group, presenting the studentship research I completed at the BDI and the work I will be undertaking for my final year university project.

Results

The cells in the blank gel appeared less discernible, with fewer distinct colonies (**Fig 1. A**). The cells in the collagen gels had many more visible colonies, these tended to be uniformly shaped in collagen I (**Fig 1. B**) and irregular in collagen III (**Fig 1. C**).

The live/dead viability assay appeared to go to plan, with most cell clusters stained green (alive), and only a few were stained red (dead) (**Fig 2.**). This shows that the cells used in the experiment were alive, ensuring the cells' normal functions and responses could be accurately observed.

The IgG stains were used as a negative control to ensure that antibody staining was specific to the collagen proteins. This helps validate that the observed staining for collagen I and III in the respective gels is accurate and not a result of background interference or antibody cross-reactivity. **Fig 3. A** indicates the presence of collagen I in the ECM around the cells due to the extensive red fluorescence. Due to the lack of red present, **B** confirms that the signal in **A** was specific to collagen I and not due to non-specific antibody binding. **C** indicates the presence of collagen III, and **D** confirms that the collagen III staining in **C** is specific and not due to non-specific binding.

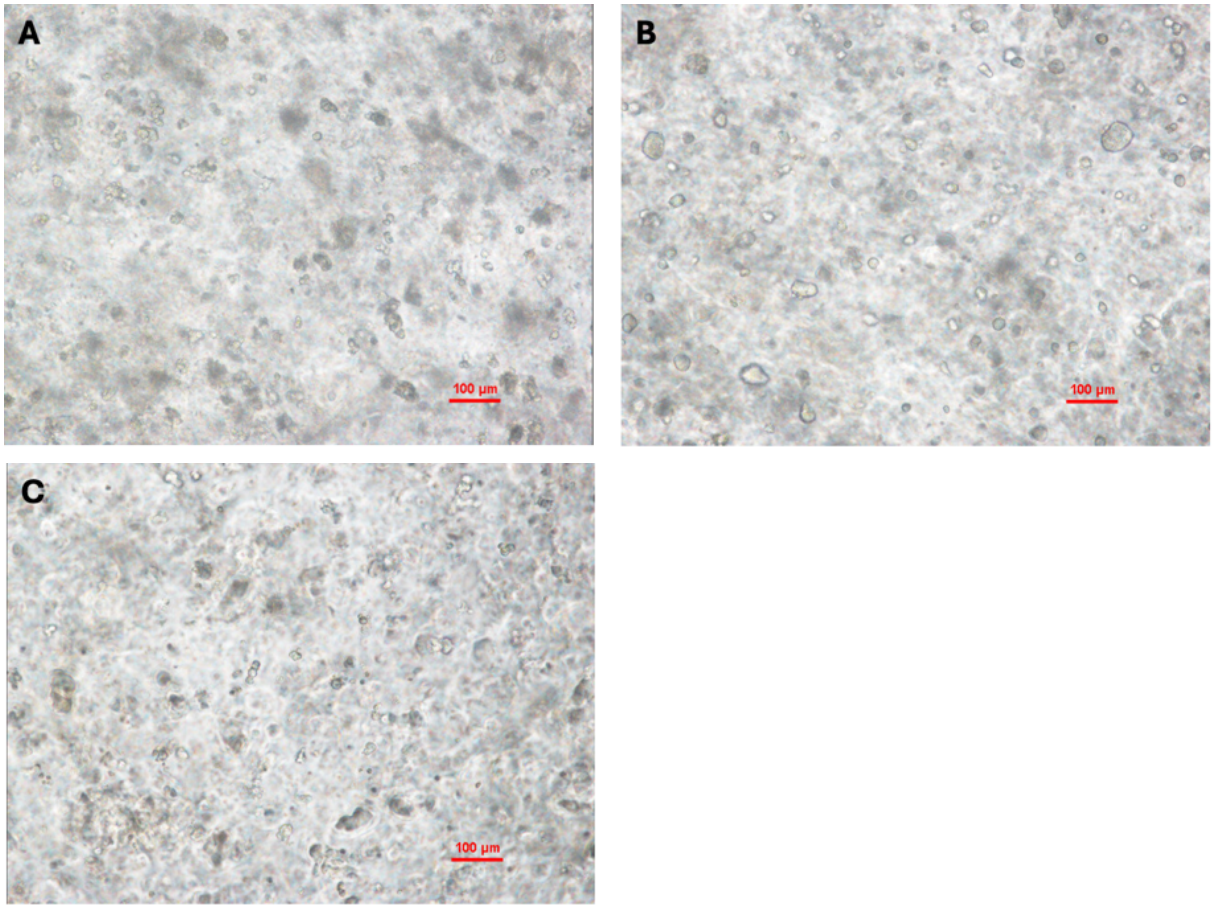

Fig 1. MCF-7 WT cells 3 days after being seeded onto well plate in hydrogel. **A** Blank gel as a control. **B** Gel with 45.8µL collagen I with many distinct uniform colonies. **C** Gel with 183µL collagen III with many irregular colonies.

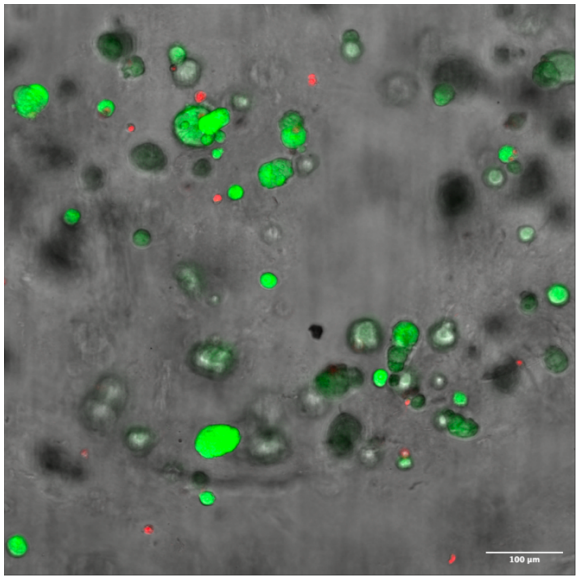

Fig 2. MCF-7 WT cells after live/dead staining observed with confocal microscope. Live cells stained with 0.5µL/mL calcein AM (green) and dead stained with 2µL/mL ethidium homodimer (red).

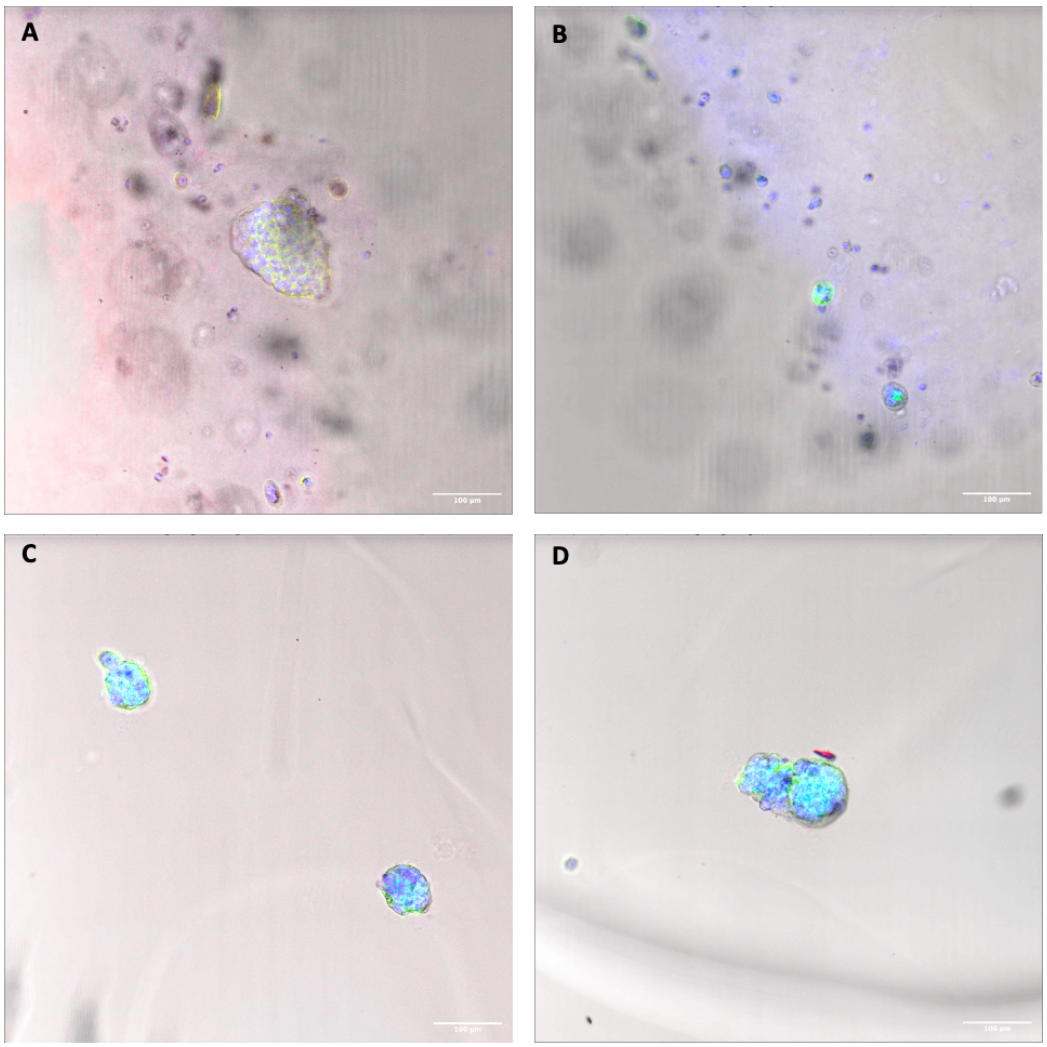

Fig 3. MCF-7 WT cells under confocal microscope. Blue = DAPI, green = phalloidin, red = collagen or IgG control. **A** Collagen I gel + anti-collagen I antibodies. **B** Collagen I gel + IgG control stain. **C** Collagen III gel + anti-collagen III antibodies. **D** Collagen III gel + IgG control stain.

Impact

The work carried out in this field has potential implications for advancing breast cancer research, particularly in understanding how collagen in the ECM contributes to disease progression. By developing a more sophisticated 3D model that mimics the ECM of breast cancer tissues, this project provides a valuable tool for investigating the dynamic interactions between cancer cells and their microenvironment. This model offers a more accurate representation of in vivo conditions compared to traditional 2D cell cultures, thus improving the reliability of findings when testing therapeutic interventions or studying cancer biology. This may also have implications in reducing the need for animal testing, as these models may be able to accurately mimic in vivo conditions.

The project's outcomes also contribute to the broader field of cancer research by providing a versatile platform that can be adapted for other types of cancer and diseases that involve ECM. Moreover, the interdisciplinary nature of the work fosters collaboration across multiple fields, including material sciences, biochemistry, and cancer biology, promoting innovative approaches to solving complex medical challenges.

In addition, the skills I developed and the training I received, such as 3D cell culture, making hydrogels, and confocal microscopy, have equipped me with a strong foundation for future research endeavours. This work also aligns with the goals of the Ashworth and Merry groups and may help contribute to future research outputs. Similarly, presenting to senior researchers allowed me to confront my fear of public speaking, while improving my presentation skills which will support my studies and future career.

Discussion

This summer project provided invaluable insights into the role of the ECM in breast cancer progression, emphasizing the importance of the tumour microenvironment in cancer biology. The 3D biomaterial models developed using PeptiMatrix technology represent a significant advancement over traditional 2D cultures, offering a more physiologically relevant platform for studying cancer-ECM interactions. These models are especially significant for understanding how cancer cells respond to mechanical cues from their surroundings, an area of growing interest in cancer research.

Overall, the project succeeded in enhancing understanding of the importance of collagen in the ECM, and with further development, it could contribute to new approaches in cancer research.