UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2024/25

NB: This whole report will be posted on the Society's website therefore authors should NOT include sensitive material or data that they do not want disclosed at this time.

Name of student:

Rhieya Rahul

Twitter Handle*:
*(*optional)*

Name of supervisor(s):

Dr Caroline Curtin

Project Title: (no more than 220 characters)

Modulation of ECM Composition to Determine the Effect on Triple-Negative Breast Cancer (TNBC) Cells in 3D Scaffolds

Project aims: (no more than 700 words)

Triple-negative breast cancer (TNBC) represents one of the most aggressive and therapeutically challenging breast cancer subtypes. Defined by the absence of oestrogen, progesterone, and human epidermal growth factor receptor 2 expression, TNBC cannot be treated with conventional targeted therapies, resulting in high rates of metastasis, recurrence, and poor patient outcomes. Emerging evidence demonstrates that cancer progression is not driven by genetic and molecular factors alone - the tumour microenvironment (TME), particularly the extracellular matrix (ECM), plays a critical regulatory role. Beyond providing structural support, the ECM delivers biochemical and mechanical signals that govern tumour cell proliferation, adhesion, and invasion, and can induce epithelial-mesenchymal transition (EMT), a process by which epithelial cells lose their polarity and adhesion properties and acquire a more migratory, invasive mesenchymal phenotype that promotes metastasis.

This project investigated how ECM composition modulation - achieved through varying collagen and hyaluronic acid (HyA) ratios in 3D collagen-hyaluronic acid (CHyA) scaffolds - influences TNBC (MDA-MB-231) cell adhesion, migration, and EMT-related changes.
Specific objectives were to:

1) Determine how ECM composition regulates TNBC cell behaviour, focusing on migration, morphology, and infiltration patterns within 3D scaffolds.
2) Assess the impact of scaffold composition on ECM remodelling over time through quantification of collagen deposition and pore size changes (surrogates for compaction and structural dynamics).
3) Evaluate N-Cadherin expression, an adhesion molecule and EMT hallmark, to explore how mechanical cues influence tumour cell phenotype and metastatic potential.
4) Correlate physical scaffold properties (collagen:HA ratio, stiffness, pore architecture) with biological outcomes (migration, adhesion, remodelling).

The rationale underlying this work stems from accumulating evidence that stiffness-driven signalling pathways, mediated by integrins and mechanotransduction, critically drive metastatic dissemination. TNBC tumours in vivo frequently exhibit regions of elevated stiffness due to increased collagen crosslinking and depleted HA content, creating microenvironments that promote invasiveness. By recreating these conditions

within 3D scaffolds, this study provides a physiologically relevant, controllable model for examining tumour-ECM interactions.
Methodologically, 3D CHyA scaffolds with varying collagen:HA ratios were engineered to mimic the mechanical and biochemical heterogeneity of the native ECM. TNBC cells were cultured for 1, 7, and 14 days to capture early, intermediate, and late-stage remodelling dynamics. Histological and immunofluorescence analyses including H&E for cellular morphology, Direct Red 80 for collagen and TRITC-conjugated N-Cadherin immunofluorescence staining was performed. Quantitative image analysis for pore size, migration distance, and collagen deposition were measured using FIJI with two-way ANOVA used to assess statistical significance. Ultimately, this project bridges material science and cancer biology by leveraging 3D biopolymer scaffolds to model tumour-ECM interactions. The findings are expected to inform future anti-metastatic strategies, highlighting how ECM composition manipulation may limit tumour invasiveness or enhance therapeutic delivery.

Project Outcomes and Experience Gained by the Student (no more than 700 words)

Project Outcomes and Impact

This project successfully elucidated how ECM composition influenced TNBC cell adhesion, migration, and remodelling within 3D environments. Although statistical significance was not achieved due to sample variability, robust biological trends emerged that provide valuable insights into tumour-ECM interactions.

Scientific Findings:

Cell Migration and Invasion: Progressive migration from Day 1 to Day 14 was observed across all scaffolds, with collagen-rich matrices exhibiting the highest and most sustained infiltration. This finding supports the hypothesis that collagen-rich ECM environments promote invasive behaviour characteristic of EMT-driven metastasis.

HyA-Rich Scaffold Dynamics: While HyA-rich scaffolds facilitated early cell infiltration, migration declined by Day 14, coinciding with the formation of rounded cell clusters. This shift likely reflects HyA degradation, scaffold compaction, and enhanced cell-cell adhesion that collectively restrict motility.

ECM Remodelling: Collagen deposition increased temporally across all conditions, with the most pronounced accumulation in control and collagen-dominant scaffolds. This indicates active ECM remodelling and alignment, both key processes in tumour stromal reprogramming.

N-Cadherin Expression and EMT Activation: Immunofluorescence revealed elevated N-Cadherin expression in collagen-rich scaffolds, particularly at early time points, signifying enhanced mesenchymal adhesion and EMT activation under increased collagen presence.

Collectively, these data demonstrate that ECM composition governs the dynamic balance between adhesive and migratory cellular phenotypes, with direct implications for metastasis modelling and therapeutic targeting.

Research Skills and Professional Development

This project provided comprehensive training in tissue engineering, cell culture, cancer biology, and analytical techniques, equipping the student with expertise in:

-Fabrication and characterisation of 3D CHyA scaffolds with tunable biomaterial compositions
-Maintenance and culture of TNBC (MDA-MB-231) cell lines under aseptic conditions

-Histological staining techniques (H&E, Direct Red 80) and immunofluorescence staining and microscopy for protein localisation
-Quantitative image analysis using FIJI for morphometric assessment (pore size, collagen density, migration distance)
-Statistical analysis using two-way ANOVA in GraphPad Prism

Beyond technical competencies, the project cultivated critical scientific thinking and experimental problem-solving. The student developed the ability to interpret complex biological trends through the lens of biophysical and biochemical principles, gaining appreciation for how ECM mechanics shape cancer pathophysiology.

Professional Skills Acquired:

Scientific Communication: Preparation of a comprehensive research poster, abstract, and oral presentation for effective knowledge dissemination.
Data Integrity and Reproducibility: Maintenance of detailed experimental protocols, structured lab notebooks, and rigorous record-keeping practices.
Collaborative Research: Close mentorship under Dr. Caroline Curtin and Dr. Eileen Reidy, fostering teamwork, professional communication, and interdisciplinary research approaches.

The project underscored the critical importance of 3D biomimetic models as physiologically relevant alternatives to conventional 2D culture for recapitulating tumour dynamics. It also deepened understanding of mechanobiology in cancer progression, an emerging interdisciplinary field at the intersection of bioengineering, cell biology and oncology.

Conclusion
This research generated meaningful insights into ECM-tumour interactions while establishing a strong foundation in experimental design, analytical rigor, and interdisciplinary collaboration. The skills and conceptual frameworks developed through this project will inform future contributions to regenerative medicine, oncology research, and biomedical innovation.

Please state which Society Winter or Summer Meeting the student is intending to present his/her poster at:

Summer Meeting

Proposed Poster Submission Details (within 12 months of the completion of the project) for an AS Winter/ Summer Meeting – (no more than 300 words)

Background:

Triple-negative breast cancer (TNBC) is an aggressive subtype characterised by the absence of oestrogen, progesterone, and Human Epidermal Growth Factor 2 (HER 2) receptors, limiting therapeutic options (1). The extracellular matrix (ECM) within the tumour microenvironment plays a crucial role in TNBC progression through biochemical and mechanical signalling. Variations in ECM stiffness, largely determined by native collagen and hyaluronic acid (HyA) composition, can modulate cancer cell invasion, adhesion, and epithelial-to-mesenchymal transition (EMT) (2).

Objective:

This study aimed to investigate how ECM stiffness, controlled by varying collagen and HyA content in 3D collagen-hyaluronic acid (CHyA) scaffolds, influences TNBC (MDA-MB-231) cell behaviour in terms of migration, collagen remodelling, and N-cadherin expression.

Methods:

3D CHyA scaffolds were developed according to established protocols in the lab (3). Scaffolds with differing collagen and HA ratios were seeded with 1X105 TNBC cells and cultured for 1, 7, and 14 days. Scaffolds were fabricated with varying collagen-to-hyaluronic acid (HA) ratios – CHyA (0.5% collagen/0.05% HyA, 0.5% collagen/0.5% HyA and 1% collagen/0.05% HyA) -to model differences in ECM stiffness and composition. Samples were fixed in formalin at experiment endpoint, prepared for histology and analysed using H&E and Direct Red 80 staining and N-cadherin immunofluorescence staining. Quantitative image analysis was conducted using FIJI, and statistical comparisons were performed using two-way ANOVA.

Results:

While no statistically significant differences were found across conditions, key trends were observed. High-collagen scaffolds promoted sustained cell infiltration and elevated N-cadherin expression, indicative of EMT-like behaviour where cancer cells become more migratory. HyA-rich scaffolds supported early infiltration but exhibited reduced migration and increased cell clustering by Day 14, likely due to matrix compaction. Pore size decreased over time across all scaffold types, reflecting ECM remodelling.

Conclusion:

ECM biochemical composition critically shape TNBC cell morphology, adhesion, and invasiveness. Collagen-enriched environments enhanced migratory and EMT-associated phenotypes, whereas HA-rich matrices transiently promote infiltration before stabilising into adhesive clusters. These findings underscore the utility of CHyA scaffolds as physiologically relevant 3D models for studying tumour-ECM dynamics and screening anti-metastatic therapies.

References:

1. Yin L et al,. Breast Cancer Research. 2020 Jun 9;22(1).
2. Monteiro MV et al. Trends in Biotechnology. 2021 Sep;39(9)
3. Sainsbury E et al. Tissue Engineering Part A. 2024 Aug 1;30(15–16):S4–S4.

Brief Resume of your Project's outcomes: **(no more than 200-250 words)**.
The title of your project and a brief 200-250 word description of the proposed/completed project. The description should include sufficient detail to be of general interest to a broad readership including scientists and non-specialists. Please also try to include 1-2 graphical images (minimum 75dpi). NB: Authors should NOT include sensitive material or data that they do not want disclosed at this time.

Modulation of ECM Composition to Determine the Effect on Triple-Negative Breast Cancer (TNBC) Cells in 3D Scaffolds

Triple-negative breast cancer (TNBC) is an aggressive subtype lacking oestrogen, progesterone, and human epidermal growth factor 2 receptors, making it resistant to targeted therapies. Increasing evidence shows that the tumour microenvironment (TME), particularly the extracellular matrix (ECM), plays a critical role in driving cancer progression. The ECM not only provides structural support but also influences how cancer cells migrate, adhere, and invade through biochemical and mechanical cues.

This project investigated how varying the composition of 3D collagen-hyaluronic acid (CHyA) scaffolds affected TNBC (MDA-MB-231) cell behaviour. Scaffolds with different collagen and hyaluronic acid ratios were fabricated to mimic distinct ECM conditions. Over 14 days, cell migration, collagen remodelling, and expression of the adhesion protein N-Cadherin were analysed using histological and immunofluorescence techniques.

The results revealed that collagen-rich scaffolds promoted greater cell migration, collagen deposition (Figure 1) and N-Cadherin expression (Figure 2), as evidenced by H&E, Direct Red 80 staining and immunofluorescence respectively, suggesting a link between collagen and invasion potential. H&E staining also showed hyaluronic acid-rich scaffolds supported early infiltration but later led to compact, adhesive clusters, reflecting dynamic ECM remodelling.

Overall, the study highlights how ECM mechanics influence TNBC progression and underscores the potential of 3D biomimetic scaffolds as powerful tools for studying metastasis and screening future anti-cancer therapies.

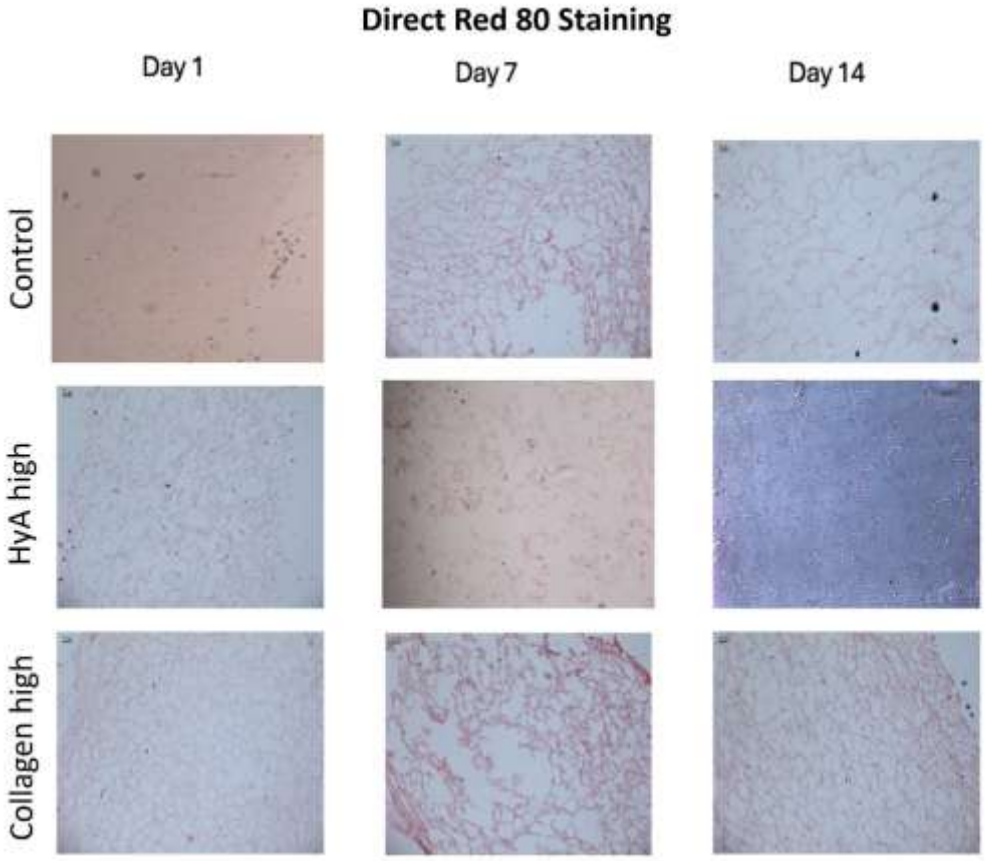

Figure 1: Direct Red 80 Stain across Day 1, 7 and 14 showed increased collagen deposition in the collagen high group, even at day 14.

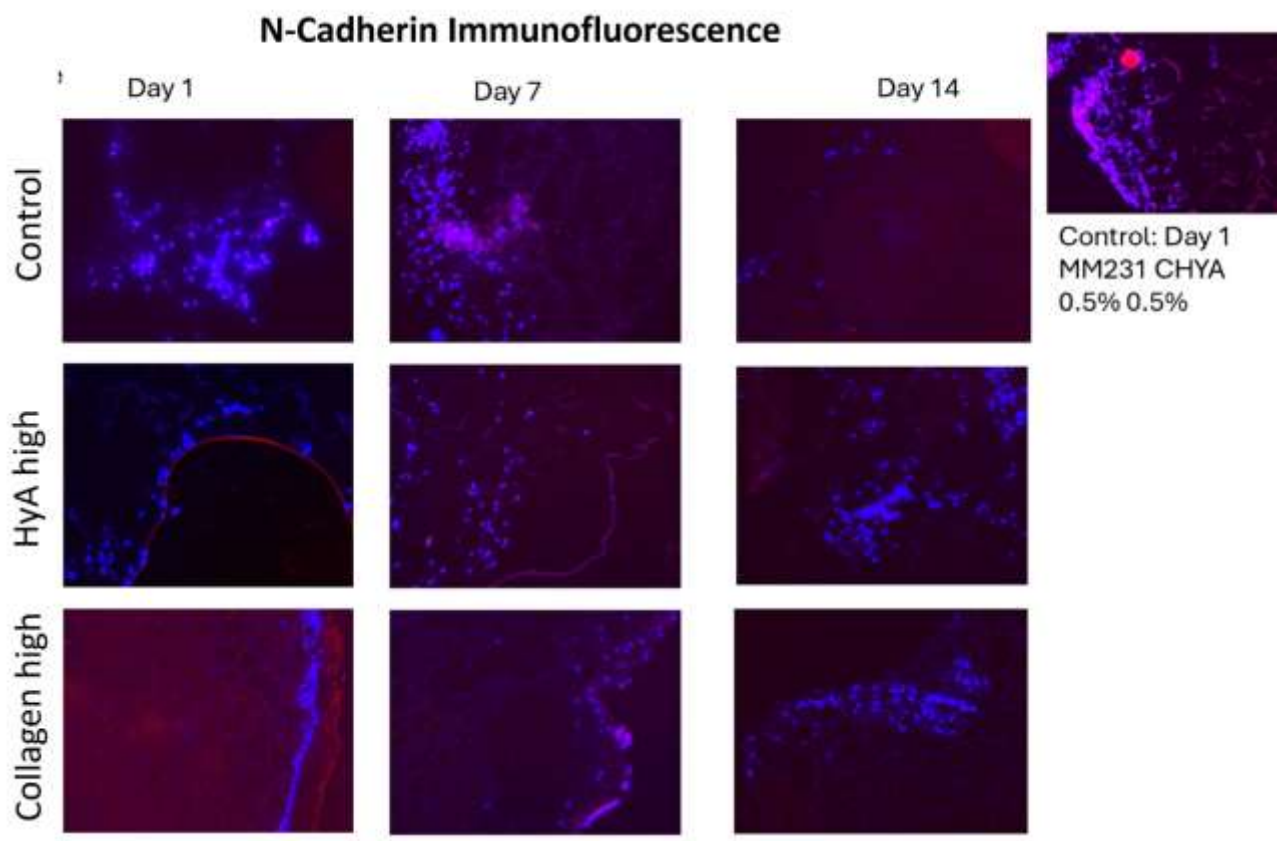

Figure 2: N-Cadherin immunofluorescence stain (DAPI (nuclei) and TRITC (N-Cadherin)) across Day 1, 7 and 14 showing enhanced staining in the collagen high group across all timepoints.

Other comments: (no more than 300 words)

Data Protection/GDPR: I consent to the data included in this submission being collected, processed and stored by the Anatomical Society. Answer YES or NO in the Box below
Yes
Graphical Images: If you include graphical images you must obtain consent from people appearing in any photos and confirm that you have consent. A consent statement from you must accompany each report if relevant. A short narrative should accompany the image. Answer N/A not applicable, YES or NO in the box below
Yes
Copyright: If you submit images you must either own the copyright to the image or have gained the explicit permission of the copyright holder for the image to be submitted as part of the report for upload to the Society's website, Newsletter, social media and so forth. A copyright statement must accompany each report if relevant. Answer N/A not applicable, YES or NO in the box below

Yes

Signature of student.....Date: 29th October 2025

Signature of supervisor… *…… Date: 29-10-2025*

END OF FORM

File: USVRS Award Letter 2025 TEMPLATE v2 APP CB 160525_Dr Caroline Curtin