UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2022/23

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:

Aoife Coffey

Name of supervisor(s):

Dr Caroline Curtin

Project Title: (no more than 220 characters)

Collagen-nanohydroxyapatite gene-activated scaffolds as treatment platforms for 3D breast cancer bone metastasis

Project aims: (no more than 700 words)

Breast cancer is the most common cancer and the second cause of cancer-related mortality amongst women after lung cancer (World Health Organisation, 2020). Triple-negative breast cancer (TNBC) is a specific subtype of breast cancer that lacks the expression of the estrogen receptor (ER), the progesterone receptor (PR) and the human epidermal growth factor receptor type 2 (HER2) (Yin *et al.*, 2020). It is one of the most aggressive subtypes of breast cancer with high mortality and metastases (Ding *et al.*, 2020). The mortality rate is 40 % within the first 5 years and 46 % of TNBC patients will have distant metastasis, in particular bone metastasis (Dent *et al.*, 2007). Concurrent with the gravity of the disease, treatment of TNBC is highly challenging. There are limited target treatment options due to the lack of receptors and as a result, chemotherapy is the main form of treatment. However, the efficacy of chemotherapy is limited by poor bioavailability, drug resistance and high toxicity in normal cells (Berrada, Delaloge and André, 2010). Currently, efforts are underway to enhance TNBC treatment with a particular focus on 3D gene delivery approaches.

MicroRNAs (miRNAs) are endogenous, non-coding RNAs that are 19-25 nucleotides in length (Kim, 2005). MiRNAs function in regulating gene expression by cleaving or degrading their target transcripts (Ding *et al.*, 2020). Many miRNAs act as tumour suppressors or oncogenes. For example, miRNA-34a induces apoptosis in cancer cells and reduces their survival rate, while miR-221 controls the activation of MDA-MB 231 and SKBR3 breast cancer cells (Ding *et al.*, 2020). MiR-146a is unique in that it acts as a tumour suppressor in some cancers and an oncogene in other cancers. Due to their ability to control the expression of genes, miRNAs have been studied to determine if they can play a role in TNBC treatment.

While miRNAs are promising forms of TNBC treatment, they are unstable and have a short half-life in the blood due to their degradation by serum nucleases (Wang *et al.*, 2016). Due to these characteristics, miRNAs require effective delivery systems to target and exert an effect on cancer cells. MiRNA-based therapeutics are providing promising treatments for TNBC, but many delivery systems have limitations including complex cell uptake, unstable and nonspecific biological distribution *in vivo*, undesired side effects and lack of targeting ability (Ding *et al.*, 2020). In the past, viral vectors were used for gene delivery, however, there are major safety concerns with this delivery system such as immunogenicity.

Non-viral vectors are becoming increasingly popular in gene delivery due to their safety, gene carrying efficiency, and their ease of mass production (Curtin et al., 2018). Lipofectamine is the current gold standard transfection reagent that is widely used in gene delivery. It has many advantages as a vector including high transfection efficiency, easy to use and it can be used in the presence of media eliminating the need to remove the media for transfection (ThermoFisher Scientific, 2023). Glycosaminoglycan (GAG)-binding enhanced transduction (GET) peptide has recently been modified to create FGF2B-LK15-8R (FLR), a gene

delivery vector (Blokpoel Ferreras et al., 2021). FLR contains three domains, a fibroblast growth factor 2 (FGF2B) heparin-binding domain, LK15 and a cell penetrating peptide (CPP) 8R.

Two dimensional (2D) models were traditionally used to study tumour growth and metastasis *in vitro*, however, these models often display different morphology, metabolic profiles, and differentiation in comparison to *in vivo* cells and lack cell-to-cell and cell-to-matrix interactions. 3D models display features that are more closely related to *in vivo* conditions. Scaffolds have been successfully used as gene-activated scaffolds for regenerative medicine purposes, for example, for bone regeneration by incorporating genes that activate pathways involved in tissue repair (Curtin et al., 2012). We hypothesise collagen-based scaffolds may be used as a treatment platform to mimic *in vivo* conditions. The overall aim of this study was to determine if collagen-nanohydroxyapatite scaffolds could be used as a gene delivery platform to inhibit TNBC cell growth.

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

During my 10-week summer studentship, I carried out a series of experiments to study collagen-nanohydroxyapatite gene-activated scaffolds as treatment platforms for 3D breast cancer bone metastasis and the effects of miR-146a transfections, using lipofectamine and GET as vectors, on the proliferation, metabolic activity and survival of breast cancer cells cultured on 3D collagen-nanohydroxyapatite scaffolds.

In preparation for the study, the collagen-nanohydroxyapatite scaffolds were crosslinked using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) to enhance the mechanical stiffness of the scaffolds. They were first hydrated by placing them in 100% ethanol. The hydrated scaffolds were transferred to an EDAC solution and left for 2 hours. Following the 2-hour period, the EDAC solution was removed, and the scaffolds washed twice in phosphate-buffered saline (PBS) to remove any remaining EDAC solution. The scaffolds were stored in PBS until use. EDAC treatment is important as it serves to crosslink collagen-nanohydroxyapatite scaffolds which decreases degradation rates, increases the denaturation temperature, and improves mechanical properties of the scaffolds.

Following preparation of the scaffolds, and in preparation for the miR-146a transfections, the scaffolds were seeded with MDA-MB-231 cells (invasive TNBC cells). Scaffolds were seeded with TNBC cells and divided into three groups: non-transfected cells (control group), Lipofectamine-miR-146a and GET-miR-146a transfected cells. An Alamar blue assay (a common fluorometric assay) was carried out on the seeded scaffolds at the timepoints day 1 and day 6 to measure the metabolic activity of the MDA-MB-231 cells (Figure 1).

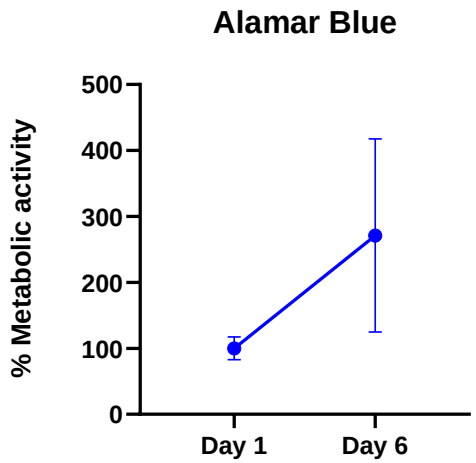

Figure 1: Alamar blue assay. The graph depicts the percentage increase of metabolic activity of the MDA-MB-231 cells on day 6 relative to day 1.

MDA-MB-231 cells were cultured for three days on the scaffolds before transfection. The non-transfected cells acted as the control group therefore only received media. The Lipofectamine and GET miR-146

transfected scaffolds were cultured for 24 hours before the transfection media was removed. An Alamar blue assay was performed on the breast cancer cells at timepoints day 1 and 6 post-transfection to determine the effect of the transfections on the metabolic activity of the MDA-MB-231 cells (Figure 2).

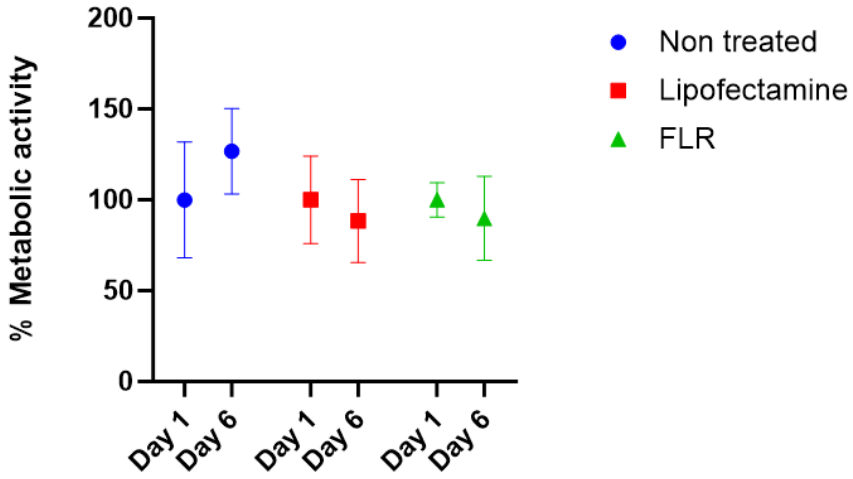

Figure 2: Alamar blue assay. The graph depicts the percentage decrease of metabolic activity of the MDA-MB-231 cells of day 6 relative to day 1 in transfected groups.

Following the Alamar blue assay, a DNA PicoGreen assay was performed to determine the DNA content in the scaffolds (Figure 3). Scaffolds transfected using the GET vector had the highest DNA content followed by the scaffolds transfected using Lipofectamine. These results could indicate that miR-146a promotes TNBC cell growth rather than acting as an antagonist. These results were also reflected in the live-/dead assay where live cells are stained green and dead cells are stained red (Figure 4). There were no dead cells present on the scaffolds transfected with miR-146a in comparison to the non-treated scaffolds indicating that miR-146a potentially enables MDA-MB-231 cells to proliferate and avoid cell death. However, based on the results in Figure 2, transfections appear to be decreasing metabolic activity as predicted. Further repetitions and extension of timepoints of this experiment are required before accurate interpretation of the data can be determined.

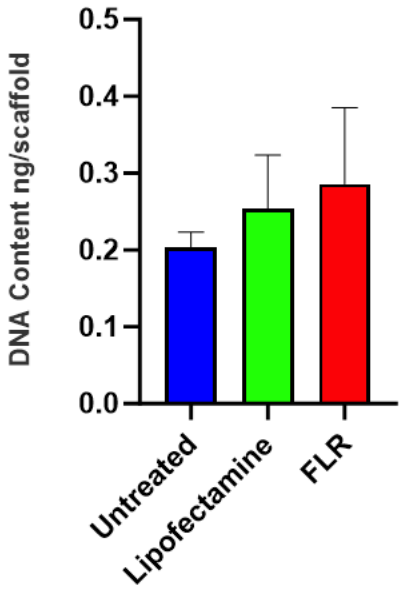

Figure 3: DNA PicoGreen assay. The graph depicts the DNA content in the scaffolds post-transfection.

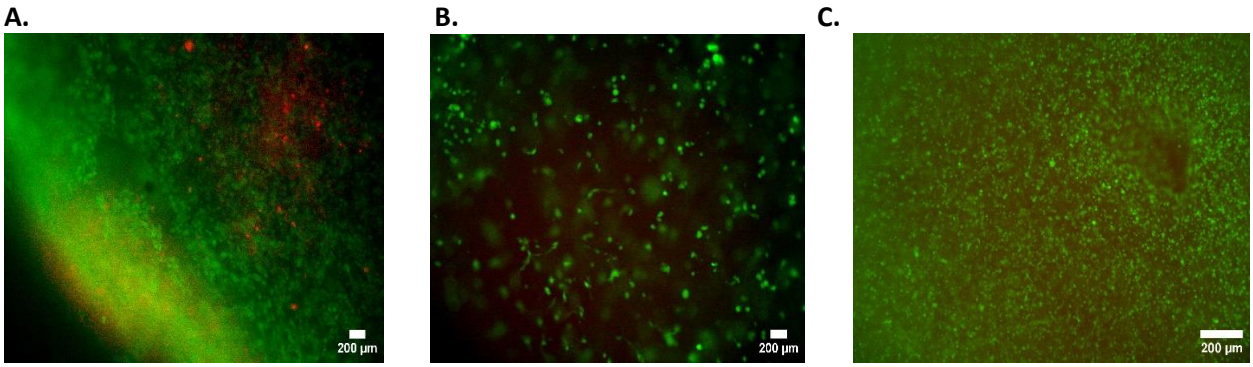

Figure 4: Live-/dead assay. Live-/dead assay of A. non-treated scaffolds, B. miR-146a transfected scaffolds using Lipofectamine, and C. GET miR-146a transfected scaffolds. Scale bar = 200 µm.

I was also involved in the setup of a breast cancer to bone metastasis study that used collagen-nanohydroxyapatite scaffolds as a platform for human mesenchymal stem cell (differentiated into osteoblasts) monocultures and co-cultures with breast cancer cells. Due to time constraints, I was not present for the entire study but was present for seeding the scaffolds, collecting the monoculture and coculture groups 21 days post-seeding and a calcium assay of the collected groups which indicated inhibition of calcium deposition in the coculture group (Figure 5).

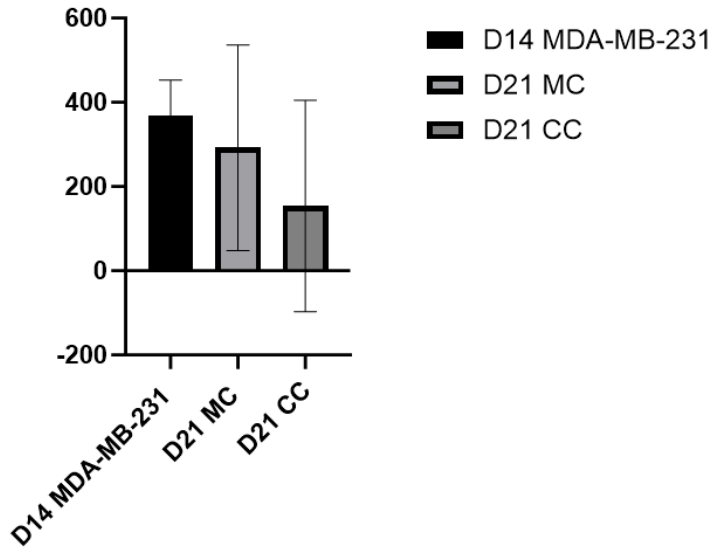

Figure 6: Calcium assay. Graph depicting the calcium deposition day 14 post-seeding MDA-MB-231 cells, day 21 post-seeding osteoblast monocultures and day 21 post-seeding osteoblast MDA-MB-231 cocultures.

This project gave me the opportunity to learn and develop a wide range of wet lab skills including cell culture, scaffold development, crosslinking protocols, seeding scaffolds, and performing Alamar blue assays, live/-dead assays, calcium assays and DNA PicoGreen assays. Additionally, I have learned many skills that will benefit me in future research such as planning and preparing experiments, analysing relevant papers to better understand my results, analysing results, plotting graphs on GraphPad Prism 10, and working with more experienced lab members to improve the project. I also prepared a draft review paper which we are currently editing for publication.

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

Summer Meeting – July 2024

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

Poster will include:

→ Background information about triple negative breast cancer, microRNAs, the vectors and 2D versus 3D cell culture models
→ Images produced using BioRender
→ Materials and methods
→ Graphs produced using GraphPad Prism 10
→Results of the Alamar blue assays, live/dead assay, calcium assay and PicoGreen assay and their significance
→ Discussion of results
→ Conclusion of project and potential future areas of research related to the project
→Acknowledgements including funding from the Anatomical Society for the Undergraduate Summer Vacation Research Scholarship
→ References to relevant literature

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.

Breast cancer is the most common cancer and the second cause of cancer-related mortality amongst women after lung cancer. Triple-negative breast cancer (TNBC) is one of the most aggressive subtypes of breast cancer with high metastases and mortality rates. Concurrent with the gravity of the disease, treatment of TNBC is highly challenging.

The aim of this study was to develop a three dimensional *in vitro* culture model to mimic breast cancer bone metastasis and determine the effects of gene delivery on the cell growth, metabolic activity, and survival of TNBC cells. To determine the effects of microRNA (miR-146a) delivery on TNBC cells, cells were cultured on collagen-nanohydroxyapatite scaffolds, transfected using Lipofectamine-miR146a and GET-miR146a and analysed for metabolic activity, DNA content and live-/dead imaging. Results indicated that miR146a has a conflicting response in TNBC, decreasing metabolic activity (Figure 1) but enhancing DNA content at the timepoints assessed. These results suggest further gene delivery experiments are warranted and extended timepoints are required to fully elucidate the action of miR146a in TNBC.

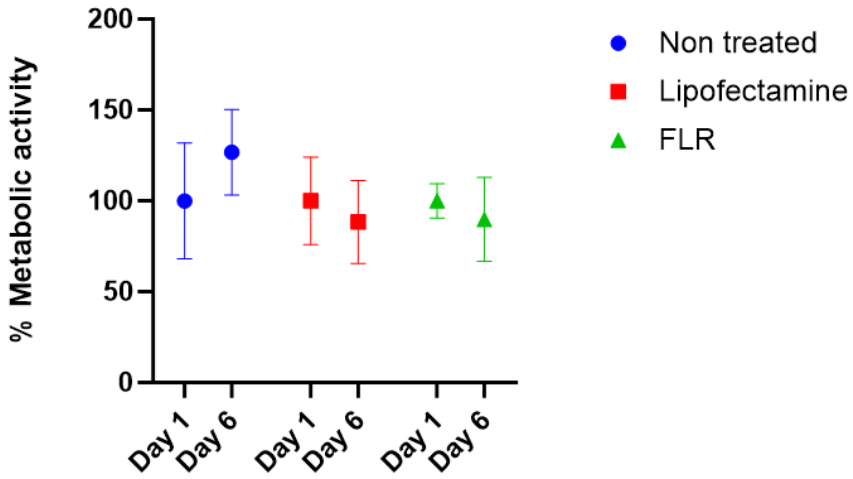

Figure 1. Alamar blue assay. The graph depicts the trend for percentage decrease of metabolic activity of TNBC cells at day 6 relative to day 1 in transfected groups.

A TNBC bone metastasis study was also assessed. Human mesenchymal stem cells, differentiated into osteoblasts, were cultured on collagen-nanohydroxyapatite scaffolds to mimic the bone microenvironment

for 7 days before addition of TNBC cells until day 21. Controls included growth of osteoblast monocultures for 21 days and TNBC cells for 14 days. Cocultures showed reduced deposition of calcium compared to controls indicating the potent effect of TNBC cells on bone formation (Figure 2).

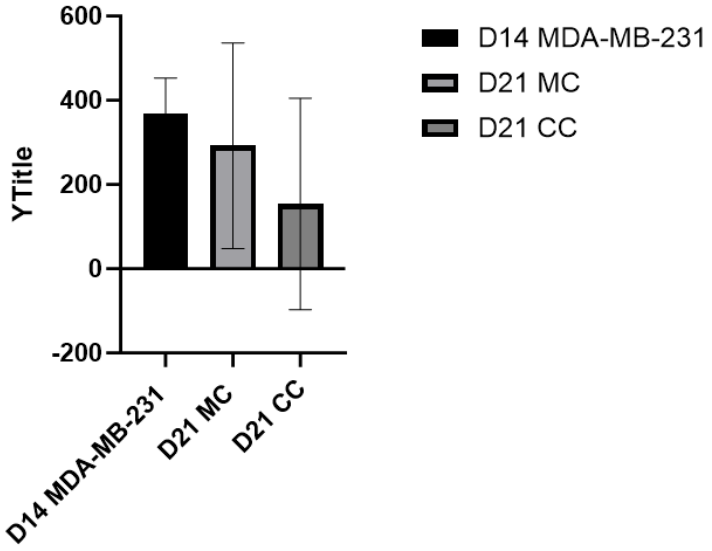

Figure 2. Calcium assay. Graph depicting the calcium deposition day 14 post-seeding MDA-MB-231 (TNBC) cells, day 21 post-seeding osteoblast monocultures (MC) and day 21 post-seeding osteoblast MDA-MB-231 cocultures (CC).

Overall, these collagen-nanohydroxyapatite scaffolds offer great potential for the assessment of novel therapies for breast cancer treatment.

Other comments: (no more than 300 words)

I would like to thank my supervisor Dr Caroline Curtin for giving me the opportunity to work in her lab, as well as Elizabeth Sainsbury, who supervised and trained me in the techniques used over the course of my summer research project. I would also like to thank Eavan Pakenham and Nezar Kama for allowing me to shadow and be involved in their projects. Finally, I would like to thank the Anatomical Society for the studentship as it allowed me to fully commit to my project and gain invaluable experience.

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Signature of student……Aoife Coffey……Date …01/11/2023……

Signature of supervisor… Caroline Curtin *…… Date…21/11/2023……*