Investigating the Propensity of Tunneling Nanotube-like Formation Between Cancer Associated Fibroblast and Pancreatic Ductal Adenocarcinoma Cells Using Live Cell Imaging Nanoscopy.

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Medical Research Honours (MEDN4001)

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Submitted: 30th October 2025 (Extension Granted)

Word Count:

8796 words

excluding Abstract, Methods and Figure Legends

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is highly lethal and resistant to therapy. One adaptation that contributes to this resilience is the formation of tunnelling nanotubes (TNTs), which enable cancer and stromal cells to exchange pro-survival cargo. This thesis investigates how two defining biophysical features of the PDAC tumour microenvironment, extracellular viscosity and cell–cell proximity, influence TNT formation. It employs the novel imaging modality rotational oblique interferometric scattering microscopy (RO-iSCAT), a label-free technique capable of distinguishing suspended nanotubes from adherent protrusions, and conceptualises two new quantitative metrics, Connectivity (the fraction of cell pairs forming connections) and Multiplicity (the number of TNTs per connected pair), which quantify TNT formation at a more granular level than conventional averaging approaches. Using these approaches, we found that TNT formation between CAFs varied with seeding density, a proxy for intercellular proximity, and that these effects were further modified under viscous conditions, indicating that TNT formation may be influenced by the physical cues of the tumour microenvironment. In addition, KPCs exposed to viscous environments adopted a highly protrusive, actin-stressed morphology compared with cells in standard media, suggesting that viscosity may modulate cytoskeletal architecture in ways that may influence their capacity to form intercellular protrusions. Collectively, this thesis associates biophysical cues of the PDAC tumour microenvironment with changes in TNT formation and establishes methodological advances that enable more precise investigation of this intercellular communication.