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Assessing the Effect of Photodynamic Therapy on Peripheral Nerve and Cancer Cells Using a Thin Tissue Engineered Collagen Culture Model

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INTRODUCTION: This study used an innovative thin 3D collagen culture system, to evaluate the response of primary rat peripheral nerve cells to photodynamic therapy (PDT). PDT is a cancer therapy that involves the administration of a photosensitive drug which becomes activated following the focal application of light to tumour sites. The subsequent production of toxic singlet oxygen results in cell death. Clinically, nerve sparing has been observed after **meta tetra hydroxyl phenyl chlorine (mTHPC)** mediated PDT [1 & 2]. This study aims to simulate nerve PDT in culture with a thin tissue engineered collagen scaffold model in order to assess the cellular basis for the phenomenon of peripheral nerve sparing after PDT.

METHODS: Mixed cultures of neurones and satellite glial cells were propagated from the dorsal root ganglia of 250-300 g rats and the human breast adenocarcinoma cell line MCF-7 was used as a comparator. Cells were seeded within 200 μ l type I collagen gels (1 mm thick discs). Cell-seeded gels were cultured for 4 days in DMEM supplemented with 10 % foetal calf serum and 1 % penicillin & streptomycin before incubation with mTHPC of various doses for 4 h. Samples were exposed to 10 min white light with a low light fluence rate (0.518 mW/cm² measured at 633 nm), and then maintained in culture for a further 24 h. Viability of cell populations was assessed using a propidium iodide (PI) exclusion assay. Neurones were distinguished from satellite cells using immunoreactivity for β III-tubulin.

RESULTS: This collagen model system supported the growth of neural and tumor cells and enabled PDT treatments to be applied in a consistent controllable manner. The collagen scaffold trapped live and dead cells throughout the staining procedures enabling microscopic analyses of treated samples (**Fig 1**). mTHPC-mediated PDT showed a cell population specific death response in the order: **MCF-7 > satellite cells > neurones** in a dose dependent manner (**Fig 2**).

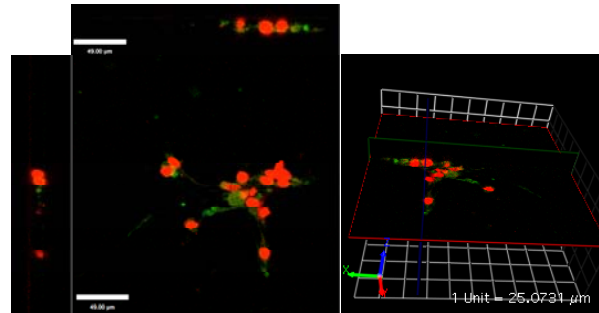


Fig. 1: Confocal micrograph showing thin 3D collagen culture model seeded with dissociated DRG culture. (Red) dead PI stained cell nuclei & (Green) β III-tubulin labelled axons.

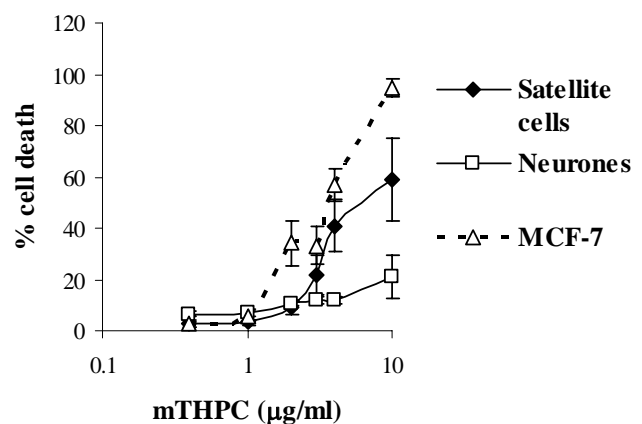


Fig 2: Dose responses of cells in thin collagen model after exposure to mTHPC-mediated PDT.

DISCUSSION & CONCLUSIONS: This model has enabled the sensitivity of different cell populations to PDT to be established. Individual cell populations were identified, and cell death defined as having PI stained (red) nuclei. These experiments indicate the possibility of a clinical PDT dose that could be used without adversely affecting neurones at the treatment site.

REFERENCES: ¹CS Betz, HR Jäger HR, et al. (2007) *Lasers Surg Med* 39(7):571-82. ²CM Moore, T.R. Nathan, W.R. Lees et al. (2006) *Lasers Surg Med* 38(5):356-63.

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