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Title: Engineering Physiologically Relevant Breast Cancer Tumour Models

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Introduction: 2D modelling of cancer cells has dominated modelling systems for drug testing and studying cellular behavior. Despite this, there are concerns over the accuracy of these models in terms of cell morphology, cell-cell interactions and cell-matrix interactions. In contrast to this animal models provide us with the complexity of growing tumours however, they lack a highly controlled environment, are costly and time-consuming. This has led to the adoption of 3D culture models to bridge the gap between 2D models and animal models. 3D models provide us with additional layers of complexity, within a controlled environment.

An attractive approach to this is the use of hydrogels which provide a cell cytocompatible environment and are capable of absorbing water, effectively mimicking the hydrated tissues of the human body. Among the different hydrogel materials, gelatin functionalized with methacryloyl (Gel-MA) has shown great potential for cancer models namely for its water-soluble activity. It is often necessary to subject Gel-MA hydrogels to UV or visible light for shape preservation. The conventional system for this process combines UV light and the photoinitiator, Irgacure 2959. However, there are concerns over the potential ability for UV light to cause DNA and tissue damage. A number of emerging visible light systems have also been produced which include the photoinitiators LAP and RuSPS that both absorb visible light and which may be more cell cytocompatible compared to the UV light system.

Aim: The aim of this study was to compare the applicability of the UV versus visible light systems to 3D breast cancer models by growing breast cancer cells within hydrogels fabricated with UV light and visible light. Understanding the potential affect of UV light on the viability, metabolic activity and proliferation of breast cancer cells is important in the process to developing a physiologically relevant model for breast cancer.

Impact: The improvement of these 3D breast cancer models will provide us with a more physiologically relevant environment to study the behavior of breast cancer cells and their interactions with adipocytes as well as a novel platform to test cancer therapeutics.

Method: MDA-MB-231, a triple-negative breast cancer cell line was successfully seeded into GelMA hydrogels crosslinked with UV+I2959 and RuSPS and LAP with visible light.

Some of the samples directly underwent swelling and mass loss analysis to determine the crosslinking efficiency of the different systems used to crosslink the hydrogels.

The rest of the gels were incubated for 14 days to allow the cells to recover from the light exposure. At 1, 3, 7 and 14 days cell viability, metabolic activity, DNA content and cell proliferation were measured.

Results: The crosslinking efficiency and the ability of the gels to swell were quantified for cell-free gels and cell-laden gels for all three photoinitiator systems. All of the systems had a comparable sol fraction (%) and mass swelling ratio within the 10 to 20 range. This shows that the

polymerization process for all systems was similar and any differences seen in cell behavior between the three systems could be attributed to exposure to UV or visible light alone.

To compare the cell behavior of breast cancer cells in the visible light and UV photo-polymerisation systems we investigated cell viability, metabolic activity and DNA content.

Over the 14 days Live-dead fluorescence images and quantification showed the cell-laden gels fabricated from all three systems demonstrated good viability (Figure 1A). There was no statistical difference between the three systems over the time period.

Consistent with cell viability, the metabolic activity and DNA content for each sample was examined to evaluate the functionality of encapsulated cells. The metabolic activity was normalized to DNA content to give the % reduction metabolic activity/ ug DNA (Figure 1B). There was no statistical difference between the three systems over 14 days. We also observed a significant drop in metabolic activity from day 1 to day 14 for all three systems indicating that the cells were becoming more comfortable and less stressed after seeding. Consistent with this the DNA content increased dramatically at day 14 for all systems.

At day 1 and day 14 some samples were sectioned and stained for ki67, a cell proliferation marker. Cells were only proliferative at day 14 and this was consistent between all three photo-polymerisation systems.

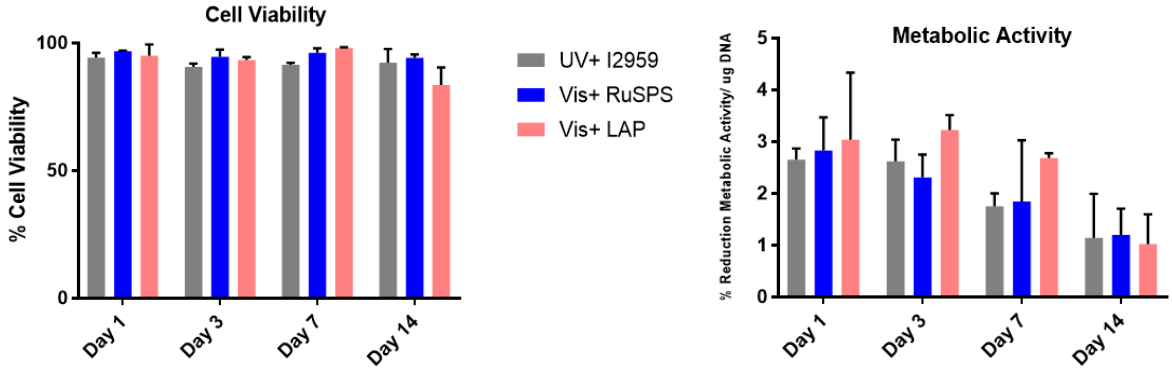

Figure 1: Encapsulation of MDA-MB-231 using UV+I2959 and RuSPS and LAP with visible light. A) Cell viability at 1, 3, 7 and 14 days. B) Metabolic Activity at 1, 3, 7 and 14 days.

Conclusion: This study demonstrates that UV is no more phototoxic towards breast cancer cells within GelMA hydrogels than visible light in terms of cell viability, metabolic activity and proliferation. This needs to be confirmed with more sensitive measures of UV-induced damage, including DNA mutation rates and measures of oxidative stress. If the findings of this study hold true, this means that the conventional UV light system stands well against emerging visible light systems to creating a more physiologically relevant environment to study breast cancer.