Multilayered-Tissue Formation Via Cell Surface Engineering and Liposome Fusion

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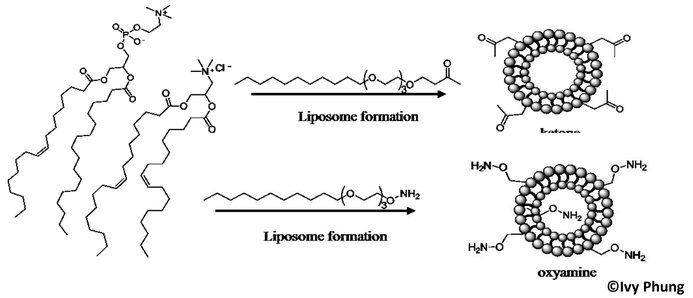
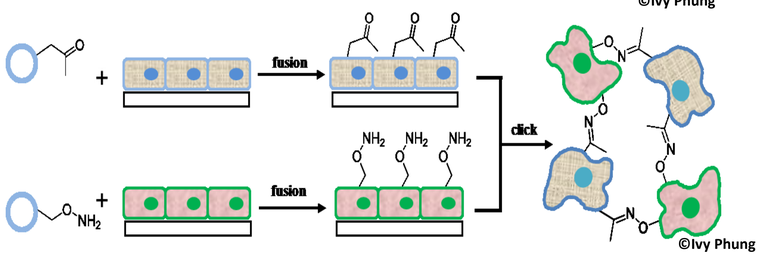
Project Goal

- Generation of multilayer connected tissues using multiple cell types in vitro and by controlling their orientation, allows for the production, re-modeling and mimicking of the complex interplay of cells in a wide range of organs in vivo.

Abstract

- A bio-orthogonal approach creating aggregates of higher order three-dimensional (3D) multilayer tissues is important to manipulate cells in combination with biologic or synthetic materials to cultivate new tissues and organs.[2]
- A previously renowned strategy for the agglutination of different cell populations into multilayers was used to create complex multilayered mechanical tissues which were then assembled and scaled with the addition of collagen

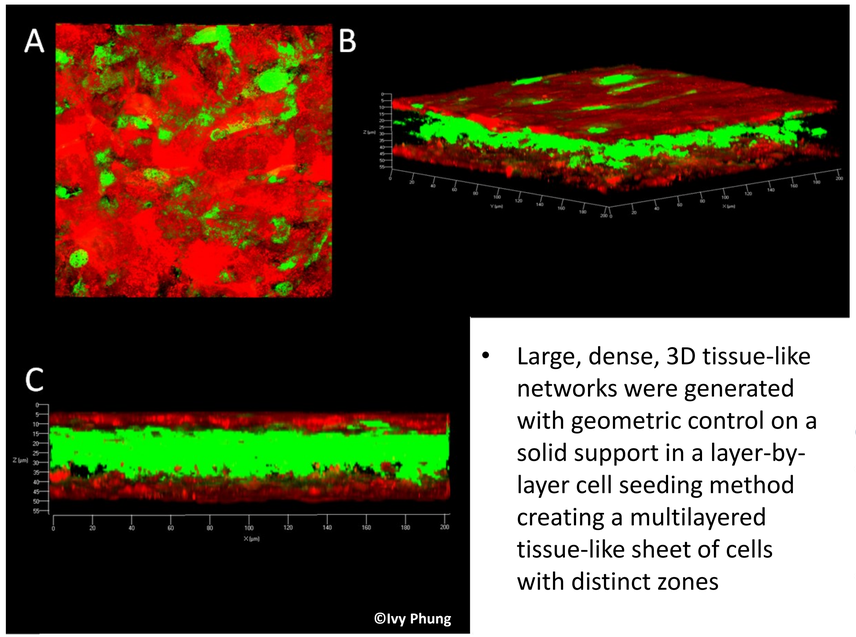




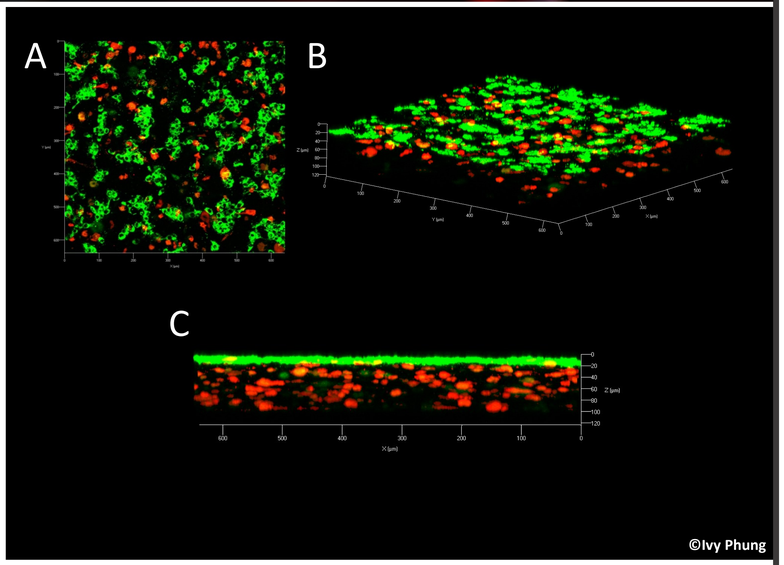
- Liposome fusion was used to introduce ketone and oxyamine functional groups to the extra cellular matrix of two different fluorescently labeled cell populations which allows for forced contact between multiple cell types and the control of the spatial and temporal arrangement of cell populations
- 3D multilayer tissues are desirable for many biomedical and pharmaceutical applications such as the production of skin grafts, generation of organs for transplantation or model organs used for drug testing and drug toxicity

Cell Adhesion for the Creation of 3D Tissue-Like Networks

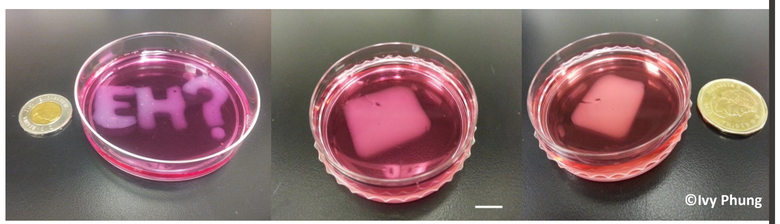
- Vesicle fusion to cell membranes was facilitated through the use of cationic lipid DOTAP, and a molecular recognition pair to induce a chemo-selective ligation resulting in cell adhesion for the production of 3D multilayer cells[1]
- Two different fluorescently labelled mammalian cell populations RFP-HNDF (Human neonatal dermal fibroblasts expressing red fluorescent protein) and NIH3T3-GFP (Swiss 3T3 mouse embryonic fibroblasts expressing green fluorescent protein) were cultured separately with ketone and oxyamine containing liposomes respectively
- Cultured cells containing subsequent functional groups were seeded on complementary surfaces resulting in an oxime reaction



- Large, dense, 3D tissue-like networks were generated with geometric control on a solid support in a layer-by-layer cell seeding method creating a multilayered tissue-like sheet of cells with distinct zones
- Oxime conjugation mediated the formation of fibroblast-fibroblast clusters creating multilayered tissues that provide a phenomenal basis for the future of bio-orthogonal tissue engineering
- Collagen, which is abundantly found in connective tissue, provides structural support and mobility while also allowing cells to access the essential nutrients needed for survival and proliferation



- Collagen incorporated with HNDF-RFP-ket cells do not interfere with cell-cell interactions while increasing structural stability



- The size and shape of tissue can be manipulated through the addition of collagen. Scale bar represents 1 cm

Conclusions

- Technique does not interfere with collagen interactions within the matrix nor with the extra cellular matrix and cell-cell interactions
- Geometrically-controlled zones of cells were fabricated in less than 72 hours
- The use of collagen provides sturdy structure for movement and ability to proliferate tissues of various shapes and sizes
- The ability to control geometrical orientation of different cell lines in a 3D multilayer complex without the confined use of scaffolds has been proven to be bio-orthogonal, rapid, chemically stable, and non-cytotoxic