An *in situ* Glucose-Stimulated Insulin Release Assay Under Perfusion Bioreactor Conditions

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Introduction

Dynamic perfusion bioreactors, unlike traditional *in vitro* cell culture systems, offer stringent control of physiological parameters such as pH, flow, temperature (T), and dissolved oxygen concentrations (DO) which have all been shown to have an impact on cellular behaviour and viability. Additionally, encapsulation of cells within a matrix exposes cells to a three dimensional (3D) environment which mimics their natural *in-vivo* one and so entice cells to behave more natively. Due to the relative infancy and the growing interest in these *in-vitro* culture systems, detection methods to assess and evaluate the function of encapsulated cells under dynamic perfusion bioreactor conditions remains one of the main challenges in tissue engineering. In this study, INS-1 cells, a rat insulinoma β-cell line which exhibit glucose-stimulated insulin release, were encapsulated in a fibrin matrix and cultured under dynamic perfusion bioreactor conditions for 24h. Cell function was assessed by quantifying glucose-stimulated insulin release.

Materials and Methods

A hollow-fibred perfusion chamber containing INS-1 cells (4 million cells/mL) embedded in fibrin (final concentration of 2 mg/mL) was introduced into a proportionally-, and integrally-controlled (PI) perfusion bioreactor and cultured for 24h. Physiological parameters were set to pH 7.4, DO 8.0 mg/mL, flow 50 mL/min, and T 37.0 °C. A glucose-stimulated insulin secretion (G.S.I.S) assay was performed to quantify insulin release. ANOVA was used to assess statistical significance.

Results

All physiological parameters were stringently controlled and maintained throughout the culture period. Phase-contrast microscopy of cells within the perfusion chamber showed a 3D morphology. Glucose-stimulated insulin release was successfully detected, quantified, and expressed per cell number.

Discussion

The dynamic perfusion bioreactor successfully maintained INS-1cell cultures embedded in fibrin, and supported viability and function of these cells. INS-1 cells appeared to interact with their fibrin matrix and remained glucose responsive throughout the G.S.I.S. This culture and detection method could be used to identify other proteins of interest using other cell types with different matrix biomaterials, whilst varying physiological parameters.

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