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A CUSTOM BUILT, MULTI-WELL CELL SCRATCH ASSAY DEVICE

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INTRODUCTION

Lipohypertrophy is a common complication of repeated insulin injection, typically presenting as dermal nodules with adipocyte hypertrophy[1]. The consequences of lipohypertrophy are not only cosmetic, the swellings often have reduced vascularity which impairs insulin absorption causing poor glycaemic control[1,2]. Glycaemic variability increases the risk of developing various health complications including retinopathy, neuropathy and nephropathy[3,4]. Although lipohypertrophy is widespread affecting in 38-64.4% of insulin injecting diabetics, its aetiology is unknown[3,4].

The objective of this project was to design and construct an apparatus to model *in vivo* 3D insulin injection in an *in vitro* 2D system, to investigate the effect of insulin injection on adipocytes, and thus, study lipohypertrophy pathogenesis. The apparatus utilises scratch assay methodology to mimic wound healing and cell migration *in vivo*. Although the technique is widespread, the method lacks standardisation, thus it is challenging to make definitive comparisons among reports in the literature, and equally difficult to compare results from the same study[5]. Thus, an additional goal of this research was to reduce the variability associated with this technique.

MATERIALS AND METHODS

An apparatus capable of creating repeatable scratches in a cell monolayer was constructed. The device pictured in Figure 1 possesses the following key characteristics:

- Creates straight scratches with smooth edges.
- Can vary its scratch depth by half the height of an adipocyte.
- The length and velocity of the scratch can be precisely controlled.

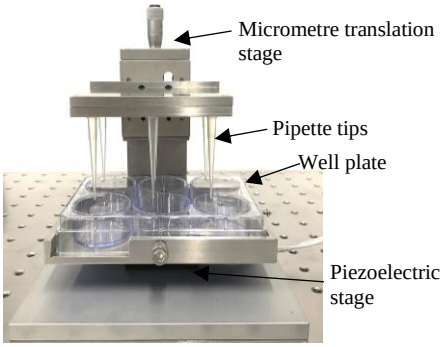

Figure 1 Developed scratch assay apparatus.

Apparatus Validation:
A preliminary investigation was conducted scratching monolayers of 3T3-L1 cells with a target scratch area of $12.5\ mm^2$ (n=6). To compare the apparatus with the state-of-the-art manual scratch by hand technique additional scratches were created with same target scratch area, with a ruler for guidance and without. The area of the wounds were calculated using microscopy software and compared to the target area. This experiment was repeated using a non-biological substance as a surrogate for the 3T3-L1 cells (n=24). The scratch areas were calculated using a custom developed MATLAB program and their variance and spread compared. The algorithm was developed to automate the tedious and error prone calculation of wound area from microscopy images using image analysis techniques through MATLAB. The algorithm was validated by comparing to experimental images from Wands et al, 2012[6] and comparing with the state-of-the-art image analysis program – ImageJ.

RESULTS AND DISCUSSION

The scratch areas were analysed, showing the apparatus created scratches with ~7 times less variation than the current state-of-the-art manual by hand technique. Using a surrogate non-biological substance, the scratches created by the device have been shown to have ~5 times less variation than manual scratches created with the aid of a ruler and ~10 times less variation than without.

The developed algorithm was visually shown to tightly line wound boundaries encompassing all appropriate cells. Additionally, the algorithm was shown to outperform ImageJ particularly when outlining partially closed wounds.

In conclusion, the custom-built apparatus has been shown to produce and analyse results more repeatably and accurately than state-of-the-art techniques.

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