Stem Cell Regeneration and Ageing Practical Write Up

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1 Introduction

Bone marrow stromal cells refer to non-haematopoietic cells that support haematopoiesis. These include mesenchymal stem cells (MSCs), which can differentiate into osteoblasts, adipocytes, myoblasts and tenocysts (Bianco et al., 2008; Burroughs et al., 1994; Kolf et al., 2007). However, an efficient method that induces osteoblast differentiation has yet been found (Castrén et al., 2015). Hence, this practical aims to develop a standardised protocol for osteoblast differentiation by comparing 2 different treatments.

Mouse bone marrow stroma cell line (M2-10B4) was cultured under fibroblast growth factor-2 (FGF-2) (Hankemeier et al., 2005) or a osteoblastic differentiation cocktail containing glycerol 2-phosphate, 2-phospho-L-ascorbic acid and dexamethasone (Langenbach and Handschel, 2013) and cultured along with controls that lack either treatments. Nonetheless, due to a lack of alkaline phosphatase (AP) staining in all replicates, no conclusions can be drawn from the results. Further research and better experimental designs will be necessary to better understand osteoblastic differentiation.

2 Results

Cells with a fibroblast-like morphology were seen in wells induced by the osteoblastic differentiation cocktail (Treatment 2) three days after the start of experiment. Three days later, similar cells were found in wells induced with FGF-2 (Treatment 1). Cells in control wells remain small and elongated throughout the experiment.

Osteoblasts display high alkaline phosphatase activity, which can be detected by staining with BCIP/NBT solution (Sabokbar et al., 1994). Cell samples were stained and photographed eight days after the start of the experiment. Images were then digitally analysed using ImageJ (Abràmoff et al., 2004; Polini et al., 2011)(Table 1).

Treatment	Measure 1	Measure 2	Measure 3	Mean	Standard deviation
Control	1572124	2269820	2281902	2040000	406000
Treatment 1	2171725	2229272	1621151	2010000	336000
Treatment 2	250835	255060	212199	239000	23600

Table 1: **Quantitative analysis of alkaline phosphatase (AP) staining.** A representative image was taken of every replicate. Images were then converted to gray-scale, before pixels were assigned colours by a 16 colour lookup table. Colours of the images were then inverted and the total number of pixels with an intensity of 90 or less was calculated for every image as an estimate for degree of AP staining.

No significant difference in staining was found between the control and treatment 1. However, there was significantly reduced staining in treatment 2 as compared to control or treatment 1 (Figure 1). This could be due to the loss of cells caused by washing cells with excessive force.

AP staining results in a strong blue-purple colouration on all cells that express AP. Hence, staining usually causes a faint but distinct background colour change (Polini et al., 2011). However, no colour change was seen during staining and none of the cells had any blue-purple precipitation when examined under the microscope. This is highly unlikely and therefore suggests that a mistake was made.

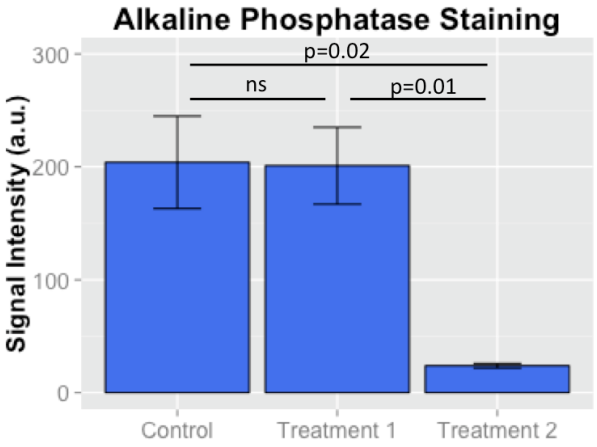

Figure 1: **Alkaline phosphatase staining.** Cell samples were stained with BCIP/NBT solution and digitally analysed using ImageJ. Intensity of staining reflects the degree of osteoblast differentiation. Treatment 1: FGF-2 (50ng/ml), Treatment 2: glycerol 2-phosphate (2.16mg/ml), 2-phospho-L-ascorbic acid (0.05mg/ml), dexamethasone (100nM).

3 Discussion

AP staining was unsuccessful and hence, no conclusions can be drawn from the results. Amount of BCIP/NBT solution required for successful staining can be tested out or a positive control of osteoblasts can be used to indicate successful staining. To prevent loss of cells, washing can be performed with a micropipette, instead of using 10 ml pipettes.

M210B4 cells have also been shown to differentiate into adipocytes but not into osteoblasts (Singh et al., 2015). It is possible that M210B4 cells are mostly adipocytes progenitors or that the osteoblast

treatment used was unsuccessful in inducing osteoblastic differentiation.

Hence, further experiments should use treatments such as a low FGF-2 concentration (3 ng/mL) (Fei et al., 2011; Hankemeier et al., 2005) and varying concentrations of ascorbic acid (Otsuka et al., 1999), dexamethasone and ß-glycerophosphate (Langenbach and Handschel, 2013) with at least three different stromal cell lines. A comparison of methods using artificial materials (Castrén et al., 2015; Kanczler et al., 2010; Polini et al., 2011) can also be performed to find the best method of generating osteoblasts from bone marrow stromal cells. As AP staining is positive for other differentiated cells, osteocalcin assays or analysis of mRNA expression levels should be used to further verify osteoblast differentiation (Arpornmaeklong et al., 2009; Polini et al., 2011).

Word Count: 595 words

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