Using CombiCult® in combination with ClonePix™ technology to select combinations of media components which induce MSC cells to secrete high levels of VEGF for therapeutic applications

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Introduction

Mesenchymal stem cells are multipotent cells derived from various adult tissues (such as bone marrow and adipose tissue) which are capable of differentiating into various cell types such as chondrocytes and osteocytes in vitro and in vivo and are used therapeutically.

Early transplantation experiments of MSCs into models of disease such as myocardial infarction (MI) showed that although the percentage of engrafted cells was low, the clinical outcomes were promising. Further investigation has revealed that these cells exert their effects through paracrine factors rather than by direct engraftment, and that they do so by secreting a plethora of therapeutic molecules that can increase tissue perfusion, decrease collagen deposition and fibrosis, enhance host cell survival and attract/regulate endogenous progenitors.

Amongst the molecules that have been found to be highly expressed by MSCs is the proangiogenic cytokine VEGF, which is responsible for formation of new blood vessels (Mazo et al. 2012). Increasing the expression of these molecules would be advantageous for the therapeutic effectiveness of MSCs . It has been proposed that this can be achieved by modulating the conditions under which MSCs cells are grown and expanded prior to transplantation.

Finding these conditions using conventional cell culture can be an arduous task as the number of possible combinations of components can be large or more complicated culture protocol involving sequential media changes may be necessary. Plasticell (Stevenage, UK) has developed a high throughput screening technology called CombiCult® (Combinatorial Cell Culture; Choo, 2008: Fig. 1) that is capable of multiplexing very large numbers of cell differentiation or maintenance media to identify optimal protocols that result in high efficiency stem cell differentiation or expansion to a given phenotype.

The goal of this experiment was to investigate the feasibility of using a secretion read-out assay in combination with a Combicult® screen to find cell culture conditions that would maximise the secretion of VEGF protein from MSCs, and to test the feasibility of using a ClonePix™ colony picker for identifying and isolating positive hits.

CombiCult® Technology

- Stem cells are seeded onto microscopic beads.
- Beads are shuffled through multiple differentiation media spiked with fluorescent tags that label the beads.
- Beads are screened to identify those bearing differentiated cells (hits).
- Hits are sorted, by a large particle sorter, into a microtitre plate.
- Tags are analysed by FACS to deconvolute the cell culture history of hits.
- Bioinformatics software ranks the differentiation protocols.

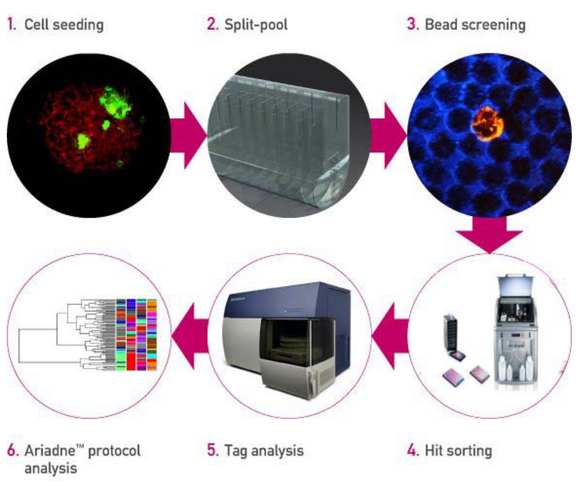

Figure 1: CombiCult® Technology.

ClonePix Technology

ClonePix™ technology allows the screening and selection of high expressing mammalian cell lines. Cells are plated at low density into viscous semi-solid media, forming discrete clonal colonies. Expressing colonies are detected in situ using fluorescently conjugated antibodies called CloneDetect (Fig.2). CloneDetect reacts with secreted target protein forming a fluorescent precipitate. The amount of fluorescence is proportional to colony productivity. High secreting plates.

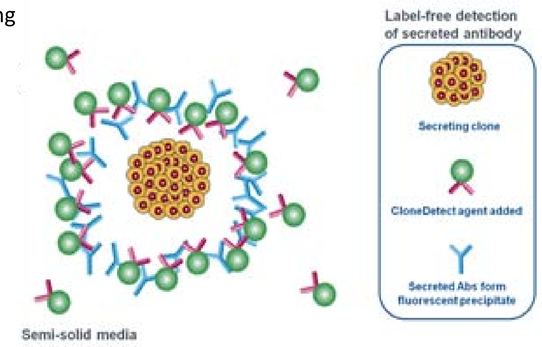

Figure 2: Detection of secreted antibody using fluorescently conjugated antibody

Method

- Bone marrow MSC cells were seeded onto 18,000 Plasticell's PTC5000 microcarrier beads in 9 different media cocktails spiked with a unique tag.
- After 4 days in culture the beads were washed, pooled and split into another 9 media cocktails, each one spiked with another unique tag.
- The cycle was repeated a third time for a total matrix complexity of 729 different combinations of media cocktails (9 x 9 x 9).
- On day 10, the beads for each condition were plated on semisolid media (with the components for each condition) containing FITC conjugated anti-VEGF antibody and incubated for a further 10 days (Fig. 3).
- After this time the beads were imaged and picked using the ClonePix™.
- The cell culture history of each picked bead was deduced by FACS analysis of the fluorescent tags attached to the bead.
- The positive bead protocols were analysed using a bespoke bioinformatics software package to identify the optimal protocols for further validation.

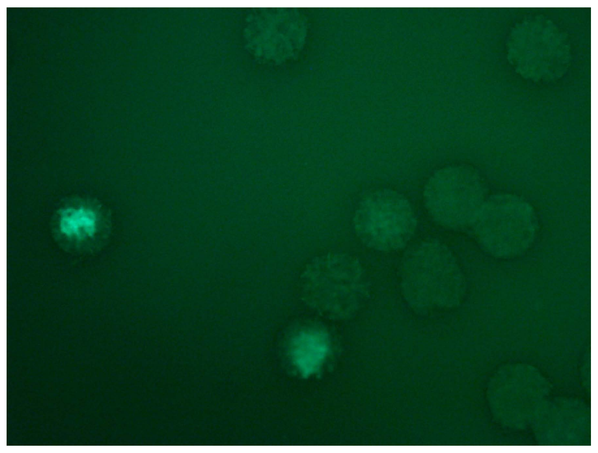

Figure 3: An example image (4x Objective) of the fluorescent labeling in the semi-solid media containing stem cell coated beads and FITC conjugated Anti-VEGF. The two brighter beads would be classed as 'hits' by Plasticell.

Imaging and Picking using ClonePix ™

Initial tests were carried out with positive and negative controls to help identify levels of associated fluorescence that were indicative of non, low and high secreting stem cell coated beads.

Beads that were of irregular shape, too large or two small to represent a bead or too close to each other for accurate picking were discarded (Fig.4).

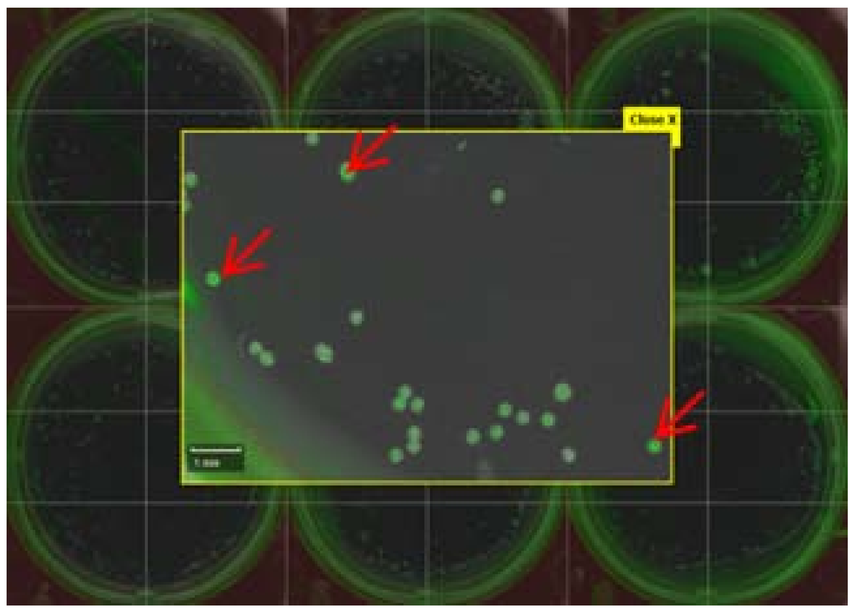

Figure 4: Composite image (White Light image with FITC overlay) of the stem cell coated beads on ClonePixTM. The zoomed in image identifies three beads to be picked (outlined in green with red arrows) that meet the specified shape , size and fluorescence criteria amongst discarded beads (no outline displayed)

The remaining group of beads were displayed on a Rank Plot and Histogram in order of Interior Fluorescence to identify those beads with higher associated fluorescence (Fig.5). A group was created to contain these beads with higher levels of fluorescence which were then selected for picking.

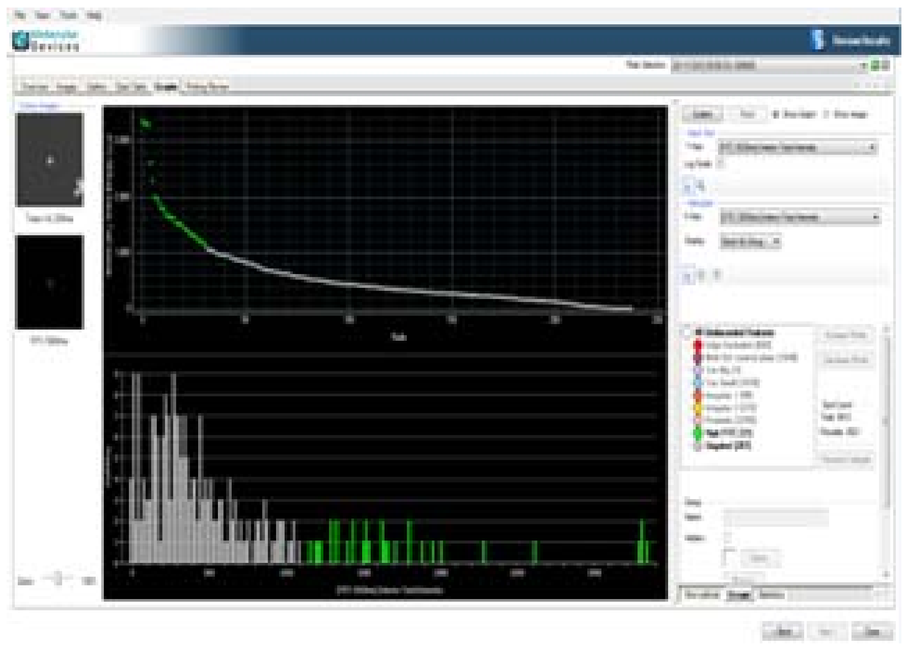

Figure 5: Rank Plot (top) and Histogram (bottom) of bead Interior Fluorescence produced by the ClonePix™ software package. Green highlighted items are the beads that were grouped for higher associated fluorescence and selected for picking.

Summary

A total of 105 fluorescent beads were detected and picked by ClonePix™. After microscopy inspection, they were analysed for tag content. From Ariadne® bioinformatics analyses (as per the CombiCult® workflow; Fig.1) it was possible to deduce the best combinations of media cocktails which increase the secretion of VEGF from MSC cells. Of the 729 possible combinations of media, 4 groups of protocols (which generate cells secreting higher levels of VEGF than standard media alone) were found. Therefore, this experiment proved the feasibility of ClonePix™ to identify and pick fluorescently labelled stem cell coated beads. Plasticell is currently optimising these protocols to develop cell culture protocols which increase the secretion of VEGF from MSCs without affecting their overall growth or phenotypic characteristics.

References

Choo, Y. (2008). Use of Combinatorial Screening to Discover Protocols That Effectively Direct the Differentiation of Stem Cells . Stem Cell Research and Therapeutics. In D. O. C. Y. Shi (Ed.), (pp. 227–250).
Mazo, M., Cemborain, A., Gavira, J. J., Abizanda, G., Araña, M., Casado, M., … Prosper, F. (2012). Adipose stromal vascular fraction improves cardiac function in chronic myocardial infarction through differentiation and paracrine activity. *Cell transplantation*, *21*(5), 1023–37.

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