

## Repair of a Wounded Heart; An Investigation of Cardiomyocyte Differentiation of Embryonic Stem Cells.

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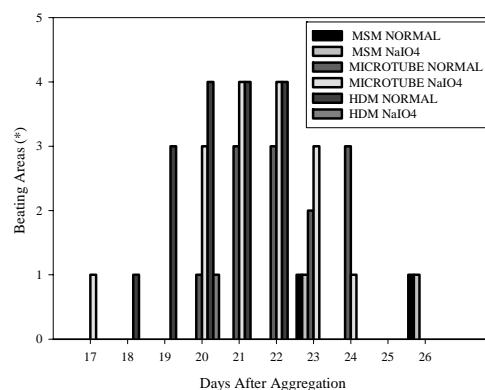
**INTRODUCTION:** Repair of heart tissue after myocardial infarction is complicated by the cells lack of regenerative capability and the need for constant activity. Insertion of a patch of ex-vivo cultured cardiomyocytes into the area of damage has shown promise, however the lack of suitable cardiomyocytes is problematic. It is foreseeable that differentiated embryonic stem cells (ESC) could be used to supply cardiomyocytes. Differentiation requires an aggregation stage, therefore a variety of methods of aggregation of stem cells for differentiation were investigated.

**METHODS:** Mouse ES14 cells were grown on a feeder layer of mouse SNL cells and maintained using DMEM supplemented with 10% FCS, 1% Penicillin/Streptomycin, 1mM L-Glutamine and 0.1mM Mercaptoethanol. Once 80% confluency was attained the cells were trypsin dissociated and differentiated by aggregation in one of the following ways; **Mass suspension:** cells were aggregated in 2 ml of media in a 6 well non-tissue culture treated plate. **Hanging droplet:** cells were aggregated in droplets of 25 $\mu$ l on the lid of a 24 well plate (each well contained 700  $\mu$ l of PBS to prevent drying; using a method modified from ref [1]). **Microtube:** cells were aggregated by settling in 1.5 ml micro-centrifuge tubes [2]. Aggregation was performed at cell concentrations of 10,000, 25,000, 100,000, 250,000, 1,000,000 and 2,500,000 cells per ml, with and without enhanced chemical binding of the cells using sodium periodate, biotin hydrazide and avidin [3].

After 2 days aggregation cells were transferred to non-tissue culture treated plates for a 5 days. They were then transferred to tissue culture treated plates and allowed to settle out and differentiate. The cells were grown for a further period of 19 days and viewed daily for areas of beating cells, using a microscope. Beating areas were measured using an estimate of the generation of beating areas, where zero indicates no beating areas, 1 is one small area of beating cells, 3 a few small areas or one medium sized area, and 5 either 1 very large area or many smaller areas of beating cells that would in total cover up to half of a well of a 6 well plate.

**RESULTS:** Beating cells were derived using all methods of aggregation; however the number and size of these differed considerably from method to method. Figure 1 shows the beating area for the different methods with and without biotin aggregation at 100,000 cells per ml. Overall, from 10,000 to 25,000 cells per ml typically generated small areas of beating cells, from 100,000 to 250,000 cells per ml large areas and/or high numbers of beating areas were seen. Above 1 million cells per ml few beating areas were seen.

**Figure 1:** ES14 cells aggregated at 100,000 cells per ml, using mass suspension (MSM), micro-centrifuge



tubes (MICROTUBE) or hanging droplets (HDM) either treated with sodium periodate (NaIO<sub>4</sub>) or left untreated (NORMAL). The beating area scale is described in the Method.

**DISCUSSION & CONCLUSIONS:** From this initial experiment it appears that aggregation by micro-centrifuge tube between 100,000 and 250,000 cells per ml will produce beating cells at about 17 days after aggregation.

**REFERENCES:** <sup>1</sup>V. Maltsev, J. Rohwedel, J. Heschler and A.M. Wobus (1993) *Mech Dev* **44**:41-50. <sup>2</sup>H. Kurasawa, T. Imamura, M. Koike et al. (2003) *J Biosci Bioeng* **96**:409-411 <sup>3</sup>P. De Bank, B. Kellam, D. Kendall (2002) *Biotechnol Bioeng* **30**:800-808

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