

Audit Trial Report: Comparison of the effects on functionality and cell differentiation of Mouse ES-derived cardiomyocytes following incubation in conventional TC-media verses AQIX® RS-I solution.

Introduction: In an independent trial conducted on mouse ES-derived cardiomyocytes, aliquots of AQIX® RS-I [Batch No. IMP/2] were utilized to assess the viability of AQIX® RS-I solution, in comparison to conventional tissue culture media (e.g., DMEM, RPMI), in terms of maintaining the spontaneous contractility of these cardiomyocytes and, more importantly, if these cells would remain differentiated throughout the trial period of 15 days.

Method: Mouse ES-derived cardiomyocytes were plated into 48-well plates containing either conventional tissue culture media or AQIX® RS-I solution and incubated at 37°C in 5% CO₂ humidified air. Both test media were replenished with fresh media every 48 hours.

AQIX® RS-I solution [Batch No.IMP/2] was stored at 3-8 °C in PE-type containers over the experimental period and had a pH of 7.25 @ RTP and pH 7.46 @ 37°C when incubated in 5% CO₂ humidified air.

Photographic and video records of the behavior of the ES-derived cardiomyocytes were obtained over the experimental period of 1 – 15 days (Figs.10 – 13).

Results; Spontaneous rhythmicity was recorded in both the ‘C’ and ‘AQIX® RS-I’ mouse cardiomyocytes after 24 hours (Figs. 10 & 11) but only for up to 9 days in the ‘C’ media whereas this activity continued unabated in the AQIX® RS-I solution for a further 6 days whereupon the experiments were concluded.

Of particular relevance was the observation that, whereas mouse ES-derived cardiomyocytes started to disappear and were replaced/overgrown by fibroblasts after Day-5 when incubated in the conventional media, those cells incubated in AQIX® RS-I solution retained both their functionality and retained their differentiated state for the 15 days studied (cf. Figs. 12 & 13).

Inference: The preliminary results indicate the pH stability, osmolality, intermediate metabolites and electrolyte composition (simulated to match that of mammalian interstitial fluid) of the serum-free AQIX® RS-I solution, was more effective than conventional TC-media in the retention of both cell-type morphology and the natural, spontaneous functionality of the mouse ES-derived cardiomyocytes.

Authors: Drs. M.Tarunina, B.Huhse & Y. Choo
Platicell Ltd
Imperial College BioIncubator
London, UK

July 4th, 2007



Figure 10. Mouse ES-cardiomyocytes in 'C' media after 1 day incubation @ 37 °C

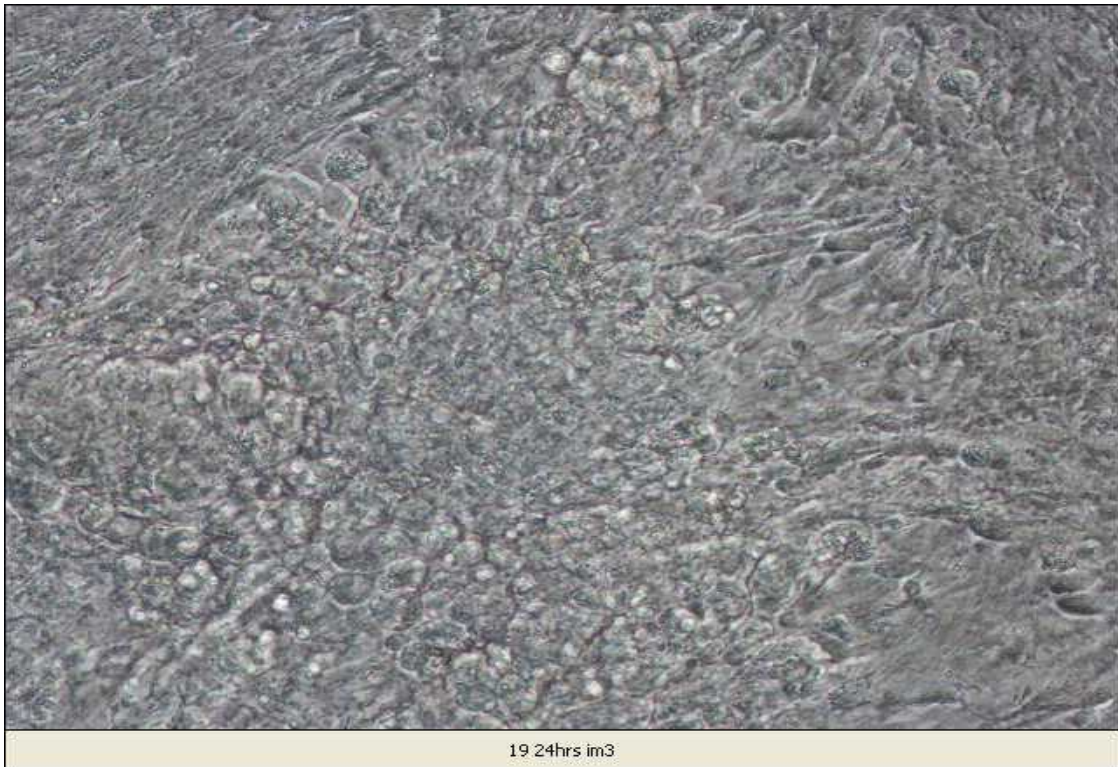


Figure 11. Mouse ES-cardiomyocytes in 'AQIX® RS-I' media after 1 day incubation @ 37 °C



Figure 12. Mouse ES-cardiomyocytes in 'C' media after 15 days incubation @ 37 °C
[Note predominance of fibroblast cells in comparison to cardiomyocytes]

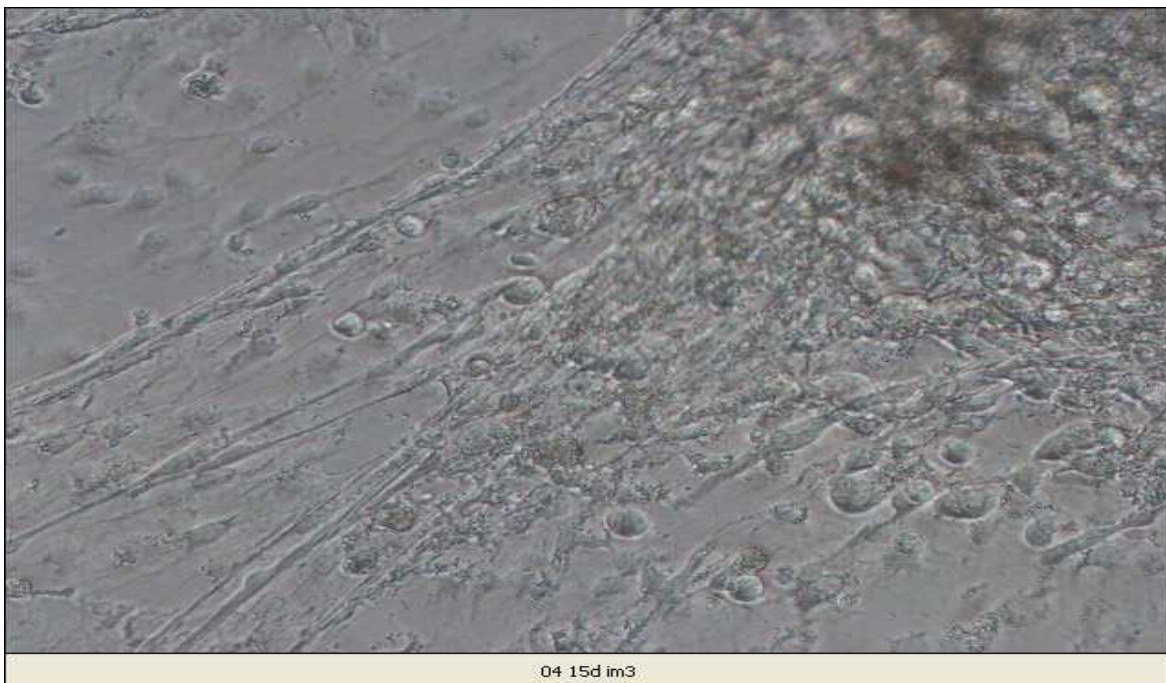


Figure 13. Mouse ES-cardiomyocytes in 'AQIX® RS-I' media after 15 days incubation @ 37 °C [Note predominance of functional cardiomyocytes]