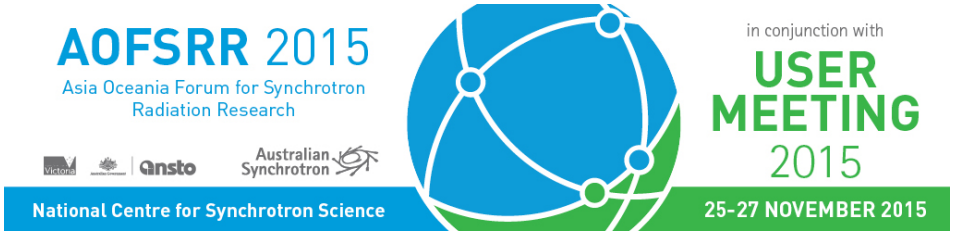

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Mechanistic insights into H9c2 differentiation of myoblasts to cardiac myocytes and skeletal muscle

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Differentiation of embryonic rat ventricular H9c2 cardiomyoblasts to cardiac myocytes and skeletal muscle has been used widely as a cell culture model system since they were characterised by Kimes and Brandt. The differentiation process and characterisation was further optimised by Mernard. Cardiac myocytes derived from H9c2 myoblasts are widely utilised to investigate cardiac muscle biology and also to study the effects cardiac hypertrophy. The cardiotoxicity as well as the protective effects of various compounds is typically explored in these cells. Despite their widespread use there is still controversy surrounding the nature of cardiac myocytes and skeletal muscle derived from H9c2 cardiomyoblasts. Therefore, we investigated the differentiation process and characterised the expression of various markers by immunofluorescence and infrared microspectroscopy. Our findings indicate that retinoic acid induces differentiation of H9c2 myoblasts to cardiomyocytes in low serum supplemented with retinoic acid. Embryonic myoblasts maintained in DMEM containing 10% FBS were cultured in DMEM containing 1% FBS for seven days resulting in differentiation to skeletal muscle. A seven day culture in low serum media supplemented with 10 nM retinoic acid resulted in differentiation into cardiac myocytes. Immunoflourescence microscopy of MLC-2v protein expression (which displays absolute cardiac tissue specificity) indicates overexpression of the protein in retinoic acid treated cells. Further, we explored chemical and elemental maps of the three different cell types and show significant spectral changes attributing to their differences. Overall, our findings indicate that the cells are well differentiated morphologically and express certain markers that are typical for each cell type.

Keywords

H9c2, embryonic myoblasts, cardiac muscle, skeletal muscle, retinoic acid

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