Novel 3D Primary Human Cardiomyocyte Culture System for Evaluation of Candidate Drug Related Cardiac Safety Profile

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Introduction

Drug discovery and development are hampered by high attrition rates that are largely attributed to the reliance on model systems that are minimally representative of the underlying human biology. Although the animal models are a good overall proof of concept, safety and efficacy evaluations in such models are hard to extrapolate to the human situation. Thus, there is an urgent need for high-throughput human *in-vitro* cell based assay systems to predict safety profile of drugs for cardiac related ailments prior to clinical evaluation. Such human *in vitro* culture systems can be utilized in mechanism-based assays for cardiotoxicity assessment. It is becoming increasingly apparent that human primary cardiomyocytes can serve as the biological and physiological relevant *in-vitro* model system for drug discovery/ cardiotoxicity screens. In this study we screened 10 potential newly synthesized small molecules: CEP2001,2002,2003,2004, 2005, 2006, 2007,2008, 2009 and 2010. These molecules were screened for their toxicity and electro-physiological activities in a 3D cardiac cell based assay. Of the 10 molecules that were screened, we found CEP2005 & CEP2010 to enhance the electro-physiological activity when compared to the untreated controls. The IC_{50} values for CEP2005 are 5ng /ml and CEP2010 25 ng /ml determined from 3D cardiac model system.

Methods:

Human Cardiac samples were obtained from consented patients at the time of open heart surgery or from automobile accident patient's hearts that were transplant reject samples. The biopsy samples were collected in Celprogen Human Cardiac Complete Growth Media M36044-15S and transported to Celprogen within 24 hours from the time of surgery. Upon receipt of the cardiac tissue samples the tissue was processed enzymatically and by mechanical dissociation prior to being plated in pre-coated 3D cardiomyocyte culture system. The cardiomyocyte cultures were maintained at 37^{0}C humidified with 5% carbon dioxide Binder C150 incubator. After 24 hours in culture the media were changed 100% and the 3D cardiac cell cultures were transferred to a C210 Binder incubator with triple gas {oxygen, carbon dioxide and nitrogen} humidified incubator equipped with IncuCyte Essen Bioscience real time imaging system. The images were obtained every hour for 72 hours. Different concentrations of the drugs were added to determine the IC_{50} values of the drugs.

The rat hearts were surgically removed and then perfuse with Rat Cardiomyocyte Complete growth media, Cat#M12117-09S, in a Langendorff system. The heart was then perfused with enzymes and left in enzymes for digestion overnight at 37^{0}C. The next day the heart tissue was mechanically dissociated and plated in E12117-09-3D-6Well plate in M12117-09S media at 37^{0}C, humidified with 5% carbon dioxide Binder C150 incubator.

The mouse hearts were surgically removed and then perfused with Mouse Cardiomyocyte Complete growth media, M11041-13S, in a Langendorff system. The heart was then perfused with enzymes and left in enzymes for digestion overnight at 37^{0}C. The next day the heart tissue was mechanically dissociated and plated in E11041-13-3D-6Well plate in M11041-13S media at 37^{0}C, humidified with 5% carbon dioxide Binder C150 incubator.

Gene Expression Profile:

Once the cell cultures were established for 72Hours, the cultures were incubated with the drugs CEP series to establish their IC_{50} values. Once the IC_{50} values were established from the 3D cardiomyocyte cultures total RNA was extracted for their Gene Expression profile. The IC_{50} values was determined by the IncuCyte Real time imager, to establish the proliferation and or inhibition rates of the test compounds and the drugs of interest.

Electrophysiology:

The human cardiomyocytes were plated in a cardiac chip for 3 days. After the third day the test compounds and drugs of interest were tested. The test compounds and the drugs were compared to the base line control values, to determine their activity.

Results:

The results are indicated in the figures and graphs below:

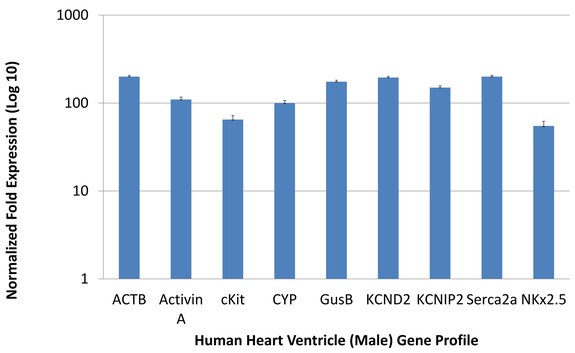

Figure 8: Gene expression profile of Human Cardiomyocyte 3D Cell Culture system

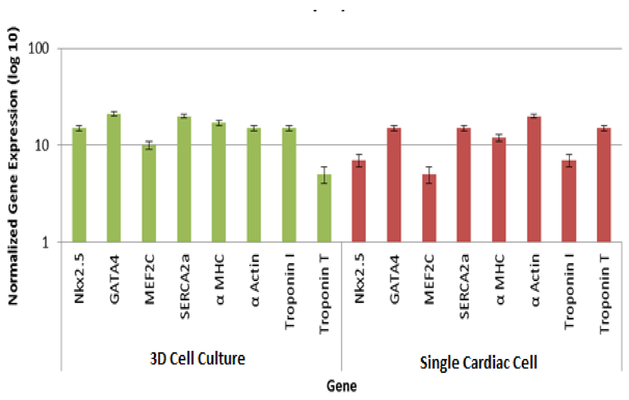

Figure 7: Gene Expression profile of single cardiomyocytes in culture and 3D cell culture

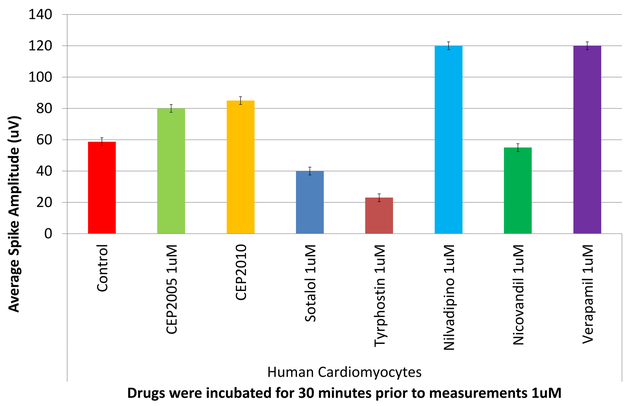

Figure 6: Electrophysiological results of test compounds and established drugs

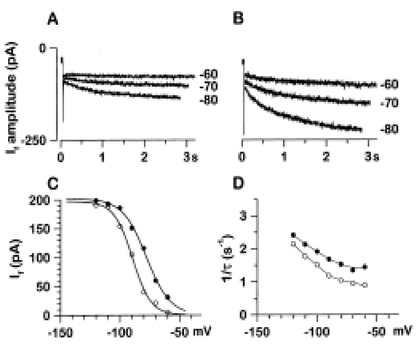

Figure 5: Electrophysiological measurement of ventricular cardiomyocyte from 3D Cell Culture

Figure 3: IC50 generation curve by Essen Bioscience IncuCyte Real Time imagerof CEP2005, calculated from 3D cardiomyocyte cell culture system

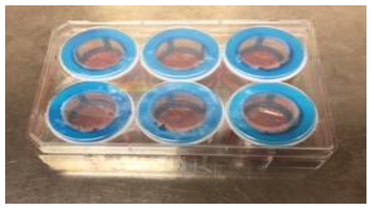

Figure 2: 3D Cardiac Cell Culture System E36044-15-3D-6Well

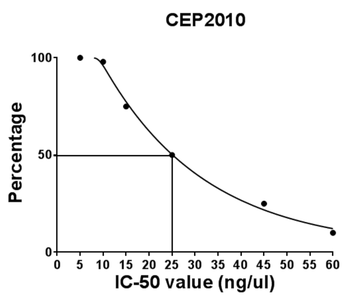

Figure 4: IC50 generation curve by Essen Bioscience IncuCyte Real Time imagerof CEP2010, calculated from 3D cardiomyocyte cell culture system

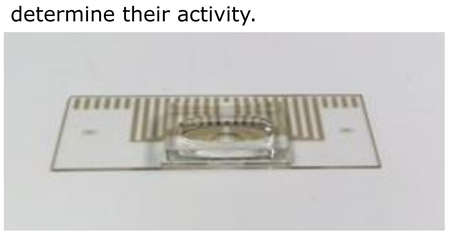

Figure 1: Cardiac Chip for measuring electrophysiology

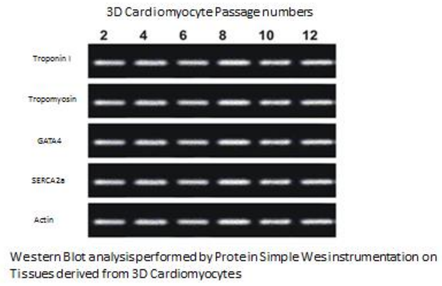

Figure 9: Protein profile of 3D Cardiomyocyte Cell Culture at different passages, performed by Protein Simple Wes, instrument.

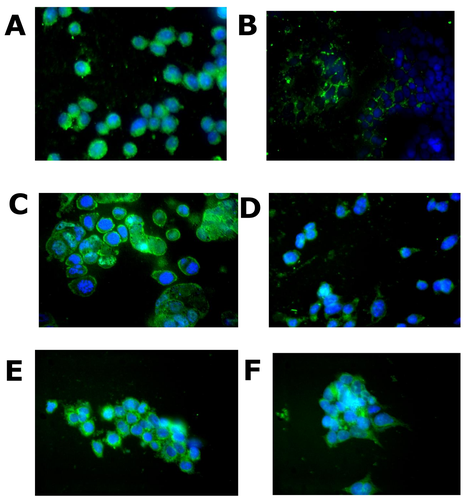

Figure 10:Single Cardiac cells .A. Actin FITC green, BrdU nuclear Blue. B. SERCA2 FITC green, BrdU nuclear blue. C. Connexin 43 FITC green, BrdU nuclear Blue. D. Troponin I FITC green, BrdU nuclear Blue. E. Tropomyosin FITC green, BrdU nuclear Blue. F. Desmin FITC green, BrdU nuclear Blue

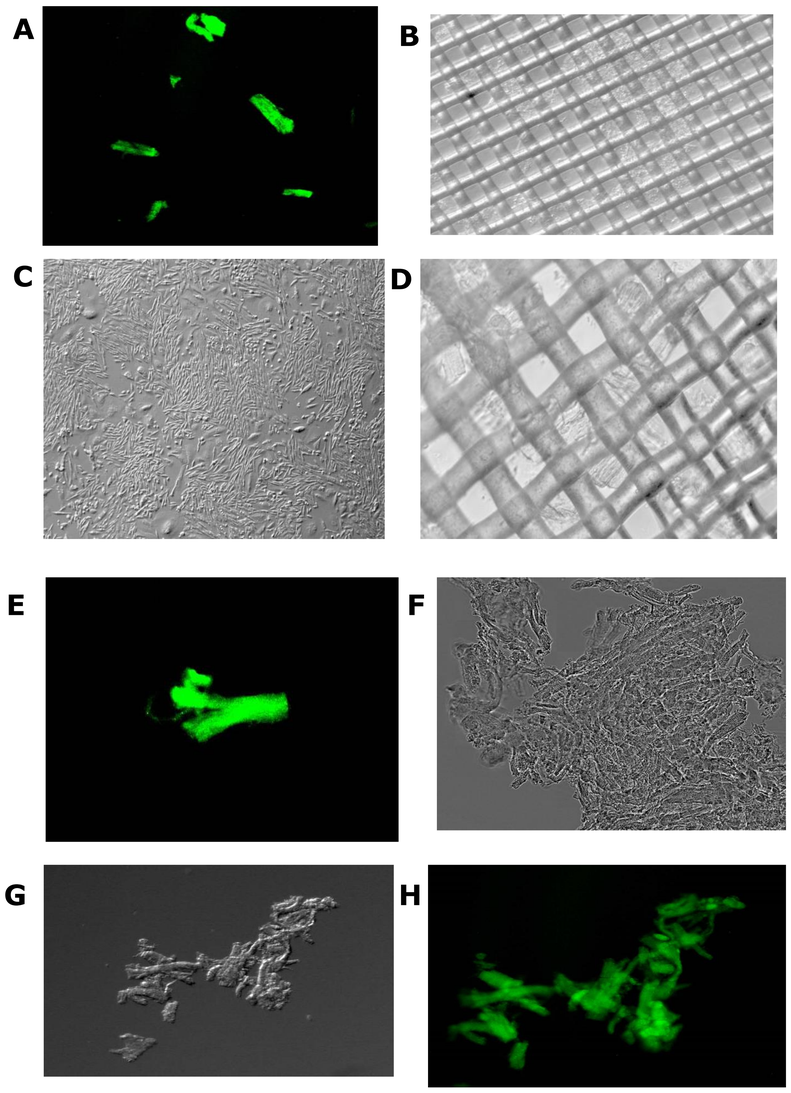

Figure 11: A. Mouse Cardiac Cell stained with Calcein. B. Mouse 3D Cardiac bright field.C. Human Cardiac single cell. D. Human 3D Cardiac. E. Human 3D Cardiac Stained with Calcein. F. Human 3D cardiac bright field. G. Rat 3D Cardiac bright field. H. Rat 3D Cardiac stained with Calcein. All the above images we obtained with Essen Bioscience, Real Time IncuCyte Imager.

Conclusions:

The newly synthesized compound CEP2005 when tested in *in-vivo* system, demonstrated an increased heart rate in mice 20% from baseline measurements. When compared to the control group both compounds CEP2005 and CEP2010 showed 15-20% increase of heart rates. From the above experiments we can conclude that we were able to screen two new compounds with the 3D culture system and determine their IC_{50} values prior to screening those further *in-vivo* systems. The results obtained from 3D culture system of new compounds were simultaneously tested with proper controls to validate the system. The following known drugs: Verapamil, Sotalol, Nicorandil, and Nilvadlpine were tested in our 3D cardiac and the electro-physiology measurement systems as positive control. Additional studies are ongoing to determine the mechanism of action for these newly synthesized compounds.

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