Improved embryoid body cryopreservation and cardiomyocyte differentiation following high hydrostatic pressure treatment

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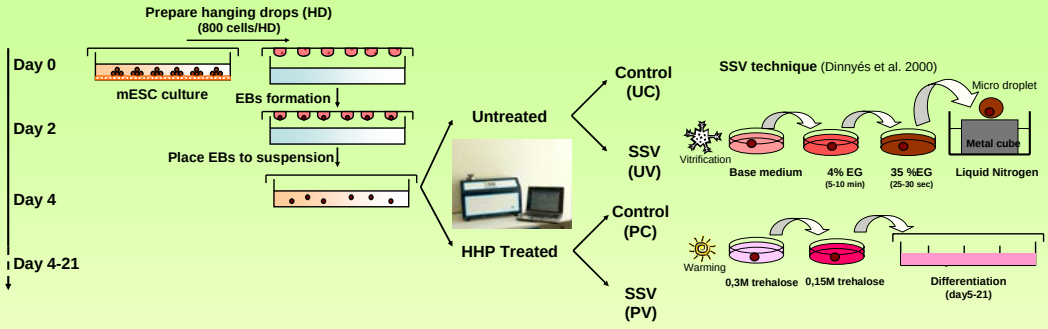
INTRODUCTION

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of blastocysts. These cells are appropriate for creation of animal models of human diseases to study gene function *in vivo.* In most protocols in order to induce differentiation of ESCs in vitro, first **Embryoid Bodies (EBs)** are produced. Therefore cryopreservation of EBs as intermediate forms prior to production of fully differentiated cells, would be highly desired. **High hydrostatic pressure (HHP)** applied as a sublethal stress treatment may induce stress tolerance of the cells. Here we report the first results of EB cryopreservation combined with HHP.

MATERIAL AND METHODS



Mouse ESCs with 129/SvJ origin were harvested with 0,05% Trypsin-EDTA and the dissociated cells were suspended in medium without LIF and hanging drops (HD) containing 800 cells were created. EBs were allowed to form for 2 days in HDs before culture in suspension for the next 2 days. On day 4 the EBs were assigned to four experimental groups (HHP treated/not treated, with or without vitrification) and 4 repetitions were made. Treatment was performed at 600 bar for 30 min at 24°C in a computer controlled pressurizing device (Cryo-Innovation Inc. Budapest, Hungary). After pressure treatment the EBs were/or not vitrified and warmed by solid surface vitrification method (SSV; Dinnyes et al., 2000), than the EBs were individually placed onto gelatin coated dishes. The survival rate were determined by observing the attached EBs. The capability of differentiation into cardiomyocytes were defined by microscopic observation of the beeting EBs and cardiomyocyte specific IHC staining.

RESULTS

Our studies showed that the pressure treatment did not affect the survival and the differentiation rates in the non-vitrified (control) groups (Table 1).

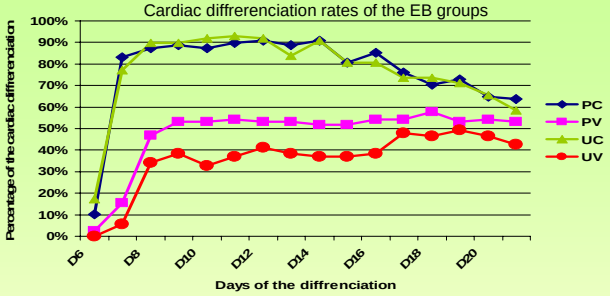
The HHP treatment had no effect on the post-warming survival rate of the EBs, however, **significantly improved the efficiency of in vitro differentiation towards cardiac lineages**. (Figure 1 and Table1)

The microscopic observation of the beeting EBs and the IHC staning are also verified the cardiac markers (Table 1).

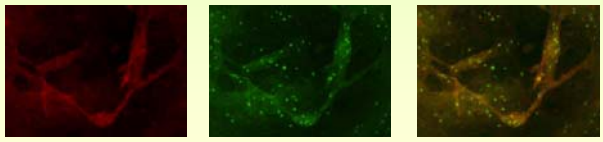
Table 1.
Survival and diffrerenciation rates of the EB groups

	Vitrified		Control	
	Untreted (UV)	HHP treated (PV)	Untreted (UC)	HHP treated (PC)
Survival rate (attached EB/plated EB)	73/88 (82,9%)	83/88 (94,3%)	87/88 (98,8%)	88/88 (100%)
Cardiomyocyte diffrentiation rate on day 14 (beetingEB /attached EB)	27/73 (36,9%)	43/83 (51,8%)*	79/87 (90,8%)	80/88 (90,9%)

Figure 1.
Cardiac diffrerenciation rates of the EB groups



Picture 1.
Cardiomyocytes specific immunfluorescens staining of the in vitro differenciated EBs



α actinin (red) GATA 4 (green) merge

DISCUSSION

In conclusion, **solid surface vitrification combined with HHP** is a highly **suitable method to cryopreserve embryoid bodies** and to improve the efficacy of cardiac differentiation from cryopreserved samples. Adaptation of the mouse model results on **human induced pluripotent stem cell** systems is ongoing at BioTalentum Ltd and expected to have a major impetus on medical application of the technology.

ACKNOWLEDGEMENT

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