Transfection Efficiency Test of Primary Human Hepatic Progenitors

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Introduction

The aim of this study was to optimize transfection efficiency of primary human hepatic progenitors. Primary cells are hard to transfect cell types. Therefore, we compared different transfection reagents for their efficiency. We used pCMV DsRed-Express2 and analyzed the percentage of positive cells by fluorescence microscopy.

Materials and methods

Total human fetal liver cells were isolated by collagenase digestion as described previously [Schmelzer et al. (2007) Human Hepatic Stem Cells from Fetal and Postnatal Donors. J Exp Med 204:1973], with slight modifications. Livers were of developmental stages between 16 and 20 weeks of gestation and were obtained as anatomical gift from the Allegheny Reproductive Health Center (Pittsburgh, PA). Organs were retrieved from abortions, after informed consent of the donor family and approval of the local Institutional Review Board. Livers cells were plated in 24-well plates for 24h in supplemented RPMI1640 including 5% FBS. Cells were plated in plastic culture ware either directly on plastic or collagen-I coated wells. After 24h cells were washed with serum-free, supplemented RPMI1640, and transfected as follows.

Experimental procedures / transfection protocol

Adherent cells after 24h in culture on plastic or collagen-1 were transfected with positive control pCMV DsRed-Express2-1 (Clontech, Mountain View, CA), negative control pUC19 (Invitrogen, Carlsbad, CA), and negative controls no plasmid and no transfection reagent. Transfection reagents included Glycofect (Techulon, Blacksburgh, VA), Metafectene Easy (Biontex, Planegg, Germany), and Effectene (Qiagen, Valencia, CA). Transfections were performed according to experimental procedures given by the manufacturer. Depending on transfection reagent, varying concentrations of plasmid DNA and reagent concentration were tested as given in the result section. 48h after transfection cells were fixed with para-formaldehyde and stained with DAPI. The percentage of DsRed-Express2-1 expressing cells was determined by fluorescence microscopy.

Results and discussion:

Transfection efficiencies for the reagents tested are given in the following tables. Conditions in tables are for one reaction in one well of a 24-well plate. All negative controls (pUC19, no plasmid, and no transfection reagent) did not result in positive fluorescence (not shown).

Glycofect:

Coating	Plasmid (µg)	**Average Positive (%)**
Collagen	1	**0.0**
	2	**0.0**
	5	**1.9**
Plastic	1	**0.0**
	2	**0.0**
	5	**8.3**

Metafectene Easy:

Coating	Plasmid (µg)	**Average Positive (%)**
Collagen	5	**25.1**
Plastic	5	**10.6**

Effectene:

Coating	Plasmid (µg)	Enhancer (µl)	Effectene (µl)	**Average Positive (%)**
Collagen	0.1	0.8	1	**8.5**
	0.1	0.8	2.5	**2.1**
	0.1	0.8	5	**4.9**
	0.2	1.6	2	**18.5**
	0.2	1.6	5	**8.1**
	0.2	1.6	10	**0.0**
	0.4	2.4	4	**12.0**
	0.4	2.4	10	**4.5**
	0.4	2.4	20	**-***
Plastic	0.1	0.8	1	**14.9**
	0.1	0.8	2.5	**5.4**
	0.1	0.8	5	**2.8**
	0.2	1.6	2	**5.8**
	0.2	1.6	5	**0.0**
	0.2	1.6	10	**1.7**
	0.4	2.4	4	**0.0**
	0.4	2.4	10	**3.8**
	0.4	2.4	20	**-***

*Cells did not survive

Conclusion / summary:

From three reagents tested (Glycofect, Effectene, and Metafectene Easy) highest transfection efficiencies of primary human fetal liver cells were obtained using Metafectene Easy in culture on collagen (25.1%). Culture on collagen did not influence negatively transfection efficiency but rather resulted in higher efficiency than in culture on plastic (10.6%).