

MEDIUM FLOW STIMULATES EXPRESSION AND ACTIVITY OF DETOXICATION GENES IN PRIMARY HUMAN HEPATOCYTES CULTURED IN A MULTICOMPARTMENT MODULAR BIOREACTOR

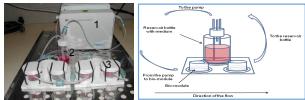
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AIM OF WORK

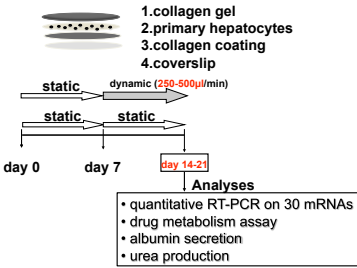
To evaluate the influence of culture medium flow on the expression of 30 hepatocyte markers in long-term cultures of **primary human hepatocytes** using a multicompartment modular bioreactor

Multicompartment modular bioreactor (MCMb) (Kirkstall Ltd. Sheffield England)

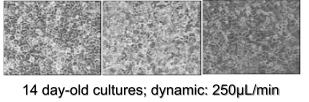


- 1. pump; 2. medium tank; 3. module of the MCMb
- flow rate (0-500µL/min)
- hepatocytes plated on collagen-coated coverslips (1.2 cm diameter), cell density 15x10⁴ cell/cm² in collagen sandwich configuration
- cell number/medium volume ratio : 15x10⁴ cells per 3 ml

Experimental protocol design

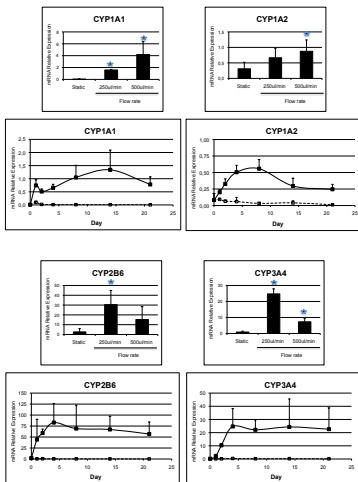


Phase contrast microscope examination of cells



14 day-old cultures; dynamic: 250µL/min

Effect of flow rate and duration of exposure to the flow on CYP gene expression



Legend

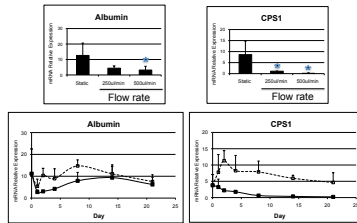
Upper panel: effect of the rate of medium flow at day 7, on mRNA expression normalized with respect to RPLP0 mRNA (arbitrary units). Lower panel: effect of time of exposure to the flow (250 µL/min). Black squares: dynamic; white squares: static conditions.

*P<0.05 with respect to static conditions; #P<0.01 for CYP1A1.

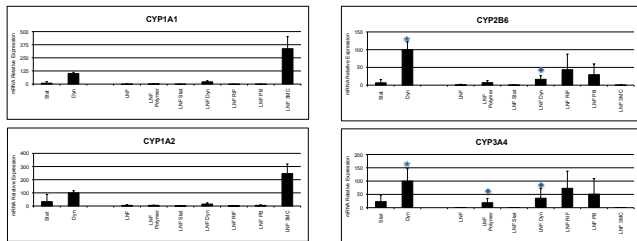
PREVIOUS STUDIES ON GENE INDUCTION BY FLOW-MEDIATED SHEAR STRESS

- In endothelial cells (venous):**
- CYP1A1 and 1B1 (Conway et al. *Cardiovasc Res* 2009; Eskin et al. *Endothelium* 2004; Han et al. *Cardiovasc Res* 2008)
 - LXR (Zhu et al. *Arterioscler Thromb Vasc Biol* 2008)
 - Kruppel-like factor (KLF2) (Dekker et al. *Blood* 2002)
 - Zinc finger protein EZF/GKLF and glucocorticoid-induced leucine zipper protein (GILZ) (McCormick et al. *Proc Natl Acad Sci U S A* 2001)
 - PAI-1 (Nakatsuka et al. *Am J Physiol Gastrointest Liver Physiol* 2006)
- In HepG2 cells:**
- CYP1A1 (Mufti et al. *Biotechnol Prog* 1996)
 - CYP3A4, UGT2B7 (Shvartsman et al. *Tissue Eng Part A* 2009)
- In rat hepatocytes:**
- CYP1A1 (Gebhardt et al. *Cell Biol Toxicol* 1996; Roy et al. *Cell Transplant* 2001)

Effect of flow rate and duration of exposure to the flow on gene expression



Effect of flow, conditioned medium and xenobiotics on gene expression



Legend

Analysis on gene expression was carried out at day 14 with a flow of 250 µL/min. **Static** (static conditions) and **Dyn** (dynamic conditions). **LNF to LNF CM**: One week-old hepatocytes were treated for 24 hours either with long term medium (LNF), conditioned media, or xenobiotic LNF polymer: medium circulated (at 250µL/min) in the bioreactor for 7 days in the absence of hepatocytes. **LNF static**: LNF medium maintained in petri dishes in the presence of hepatocytes for 7 days (static control). **LNF dyn**: medium circulated (at 250µL/min) in the bioreactor for 7 days in the presence of hepatocytes. **LNF RQ**: LNF PB and LNF 3MC: hepatocyte cultures treated with 25 µM roflumetinol, 1 mM phenobarbital, or 5µM 3-ethylcholanthrene, respectively. For each individual gene, the average ratio of mRNA expression to RPLP0 mRNA (arbitrary units, n=3) is presented. *P<0.05 with respect to LNF Polymer and LNF Dyn; #P<0.05 with respect to LNF.

Effect of flow on hepatocyte gene expression

Gene	Dynamic	Static	Sandwich	Collagen	1H8	Hep2C23
Albumin	249 ± 429	816 ± 541	2727 ± 671	1219 ± 206	1000 ± 753	6251 ± 834
AL1	96.12 ± 89.66	2119 ± 1706	92.36 ± 42.76	1622 ± 24.38	1000 ± 47.76	4497 ± 349
Albumin V	15.44 ± 1.54	157.4 ± 4.78	63.99 ± 25.6	15.45 ± 4.61	1000 ± 52.35	4177 ± 628
VH8	7.83 ± 4.48	5.78 ± 4.53	14.62 ± 5.95	5.90 ± 3.53	1000 ± 41.76	639 ± 601
CYP1	15.31 ± 14.98	54.26 ± 43.14	53.69 ± 36.73	26.30 ± 8.47	1000 ± 78.59	6.49 ± 8.87
CYP2	5.22 ± 4.87	672 ± 575	9.45 ± 4.29	2.64 ± 2.39	1000 ± 53.80	703 ± 76
Hep2C1	2.62 ± 4.14	1.26 ± 1.89	2.94 ± 2.33	1.26 ± 0.72	1000 ± 27.50	6.87 ± 8.81
CN	2.79 ± 6.73	2.89 ± 2.86	12.37 ± 4.48	13.89 ± 15.94	1000 ± 63.82	6.12 ± 7.6
HPL4	2.66 ± 2.88	4.86 ± 4.42	13.74 ± 16.47	15.45 ± 16.61	1000 ± 45.79	1.88 ± 4.55
Appl1	6.77 ± 8.71	1.49 ± 2.20	4.84 ± 2.42	2.14 ± 1.85	1000 ± 41.76	6.48 ± 8.83
Appl2	2.56 ± 1.62	4.48 ± 3.12	7.83 ± 4.99	5.70 ± 3.82	1000 ± 42.85	2.84 ± 3.8
HFN4	82.51 ± 21.73	7816 ± 28.95	97.83 ± 42.76	7816 ± 28.95	1000 ± 64.81	24237 ± 526
CYP3A4	29.19 ± 3.39	29.87 ± 16.45	83.83 ± 25.29	33.83 ± 19.45	1000 ± 56.14	16.87 ± 2.58

NQ: Not Quantifiable. NC: Not Calculable. *p<0.05 with respect to gene expression in dynamic conditions.

Effect of flow on expression of detoxication genes

Gene	Static	Dynamic	Static	Dynamic	Static	Dynamic	Static	Dynamic	Static	Dynamic
CYP1A1	100.00	100.00	22.22	22.22	36.36	36.36	54.54	54.54	72.72	72.72
CYP1A2	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
CYP2B6	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
CYP3A4	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
CYP3A5	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A1	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A2	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A3	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A4	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A5	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A6	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A7	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A8	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A9	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A10	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A11	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A12	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A13	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A14	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A15	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A16	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A17	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A18	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A19	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A20	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A21	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A22	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A23	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A24	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A25	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A26	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A27	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A28	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A29	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44
UGT1A30	100.00	100.00	11.11	11.11	22.22	22.22	33.33	33.33	44.44	44.44

NQ: Not Quantifiable. NC: Not Calculable. *p<0.05 with respect to gene expression in dynamic conditions.

Effect of flow on CYP-mediated monooxygenase activities

conditions	hydroxycoumarin	4-hydroxycoumarin	4-hydroxyphenol	4-hydroxyphenol
F330 static	0.812	0.824	0.813	0.813
F330 dynamic	0.802	0.813	0.813	0.813
F330 static	0.802	0.813	0.813	0.813
F330 dynamic	0.802	0.813	0.813	0.813
F330 (mean)	0.8057±0.035	0.816±0.037	0.816±0.037	0.816±0.037

n activities are in nmol hour⁻¹ cell. n=30 for hydroxycoumarin 4-hydroxylation and midazolam 1-hydroxylation, and n=10 for 4-hydroxyphenol 4-hydroxylation.

n=30 for hydroxycoumarin 4-hydroxylation and midazolam 1-hydroxylation, and n=10 for 4-hydroxyphenol 4-hydroxylation.

n=30 for hydroxycoumarin 4-hydroxylation and midazolam 1-hydroxylation, and n=10 for 4-hydroxyphenol 4-hydroxylation.

CONCLUSION

Primary human hepatocytes respond to flow by an increased expression of several genes involved in the detoxication function. The designed MCMb could therefore be used to study drug metabolism and toxicity *in vitro* in more physiological conditions for prospective pharmacological or pharmacokinetic studies.