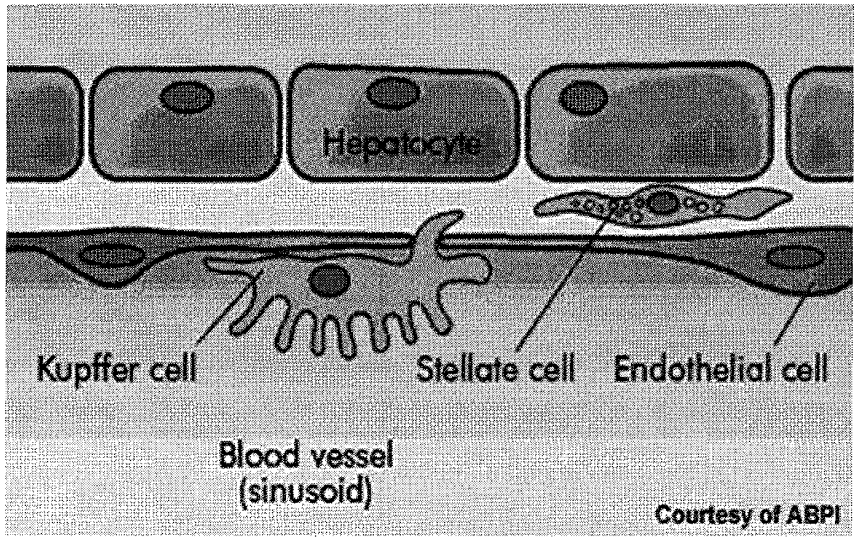
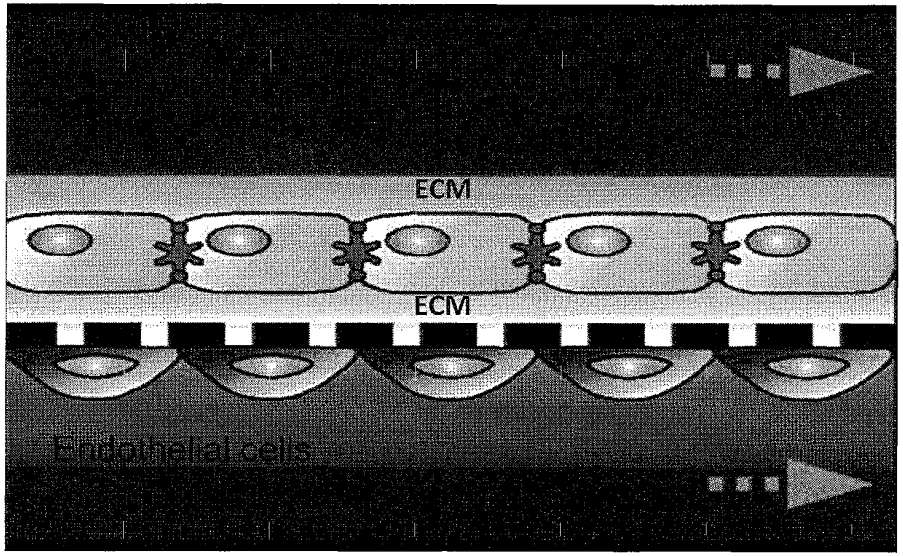
Figs. 1A-1B

A



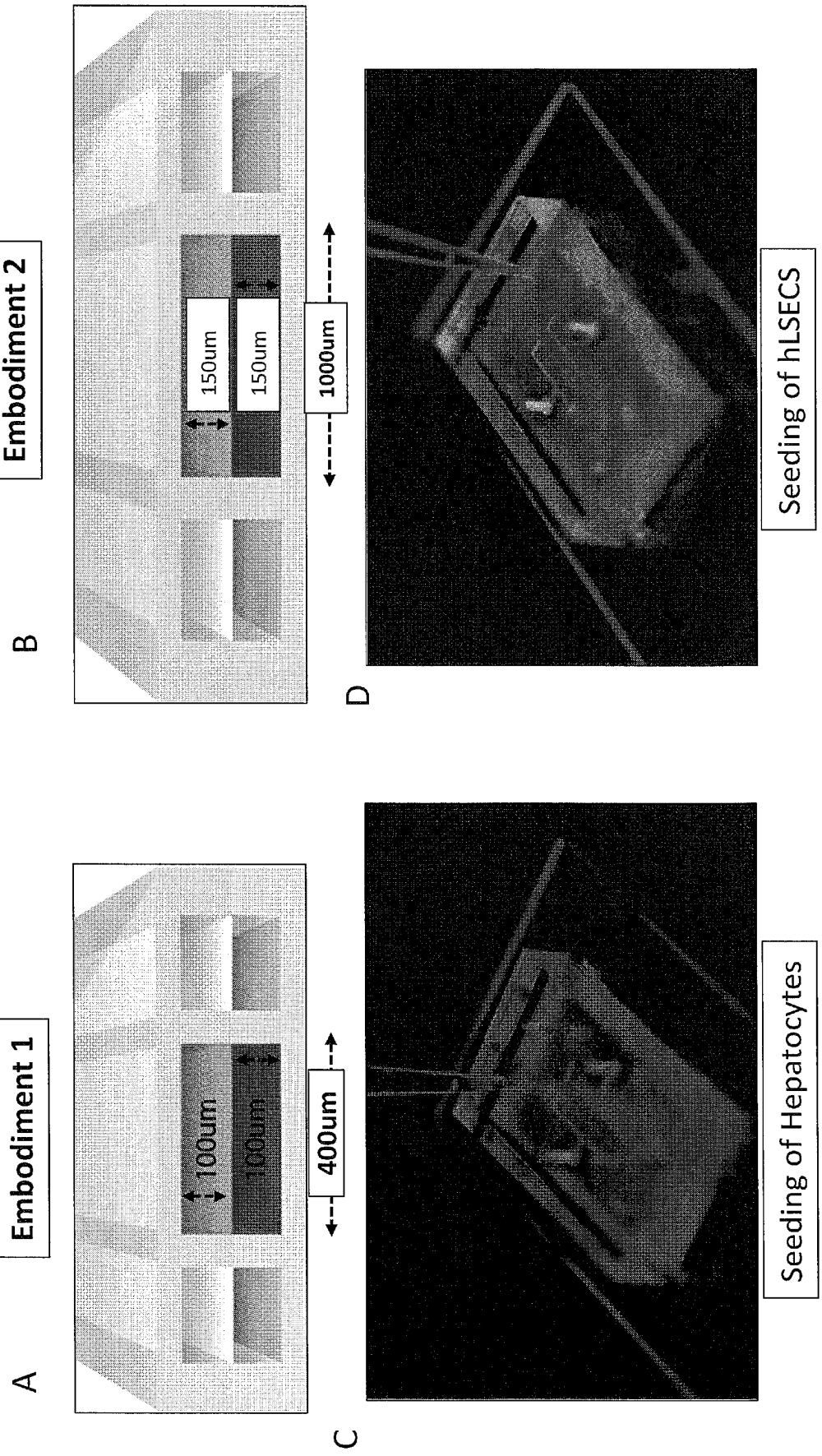
Schematic of Human Liver Structure

B



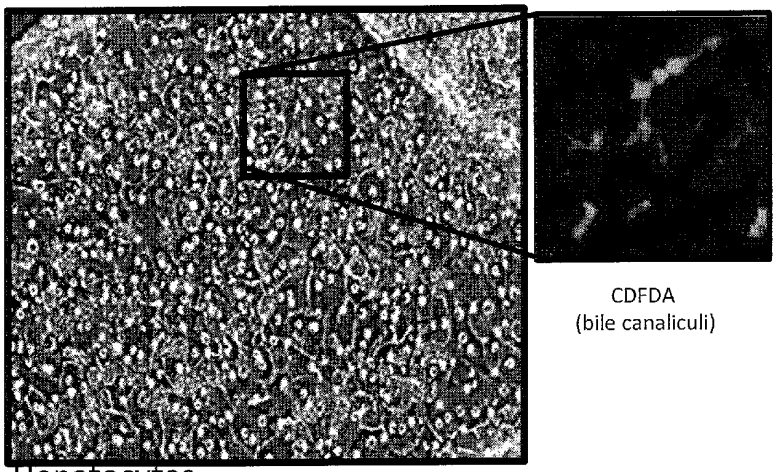
Liver-on-a- Chip Design

Figs. 2A-2D

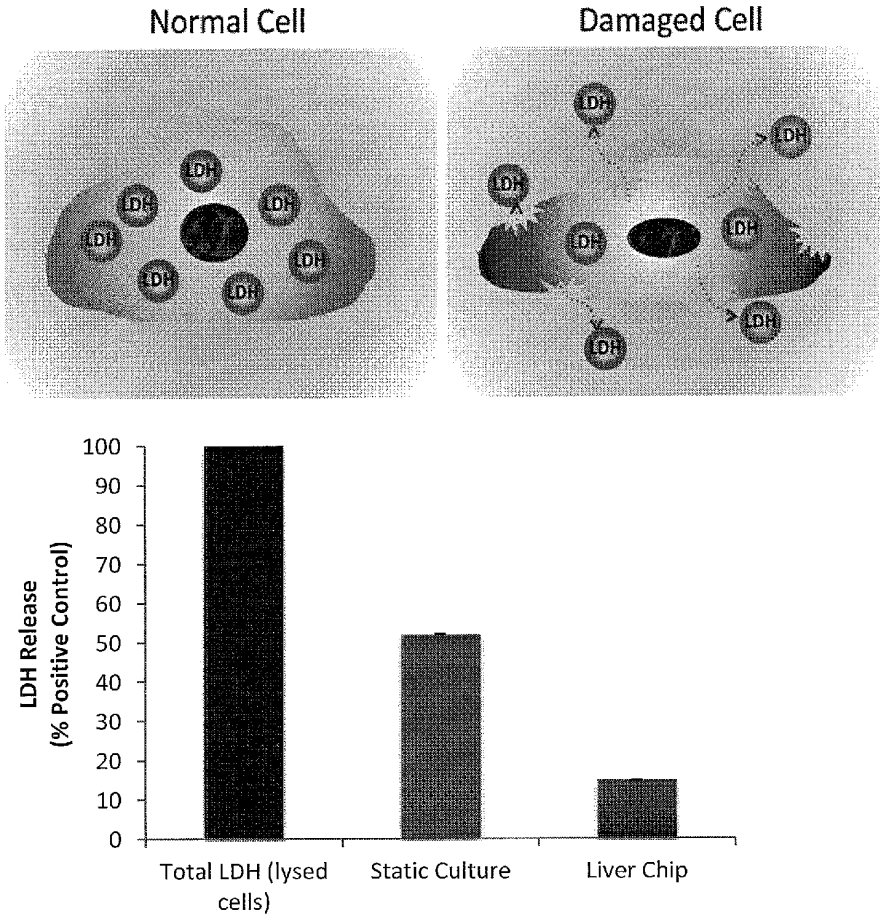

A
Embodiment 1
100um
100um
400um
B
Embodiment 2
150um
150um
1000um
C
Seeding of Hepatocytes
D
Seeding of hLSECS

Figs. 3A-3B

A Morphology



B Cell Viability (LDH Release)



Figs. 4A-4B

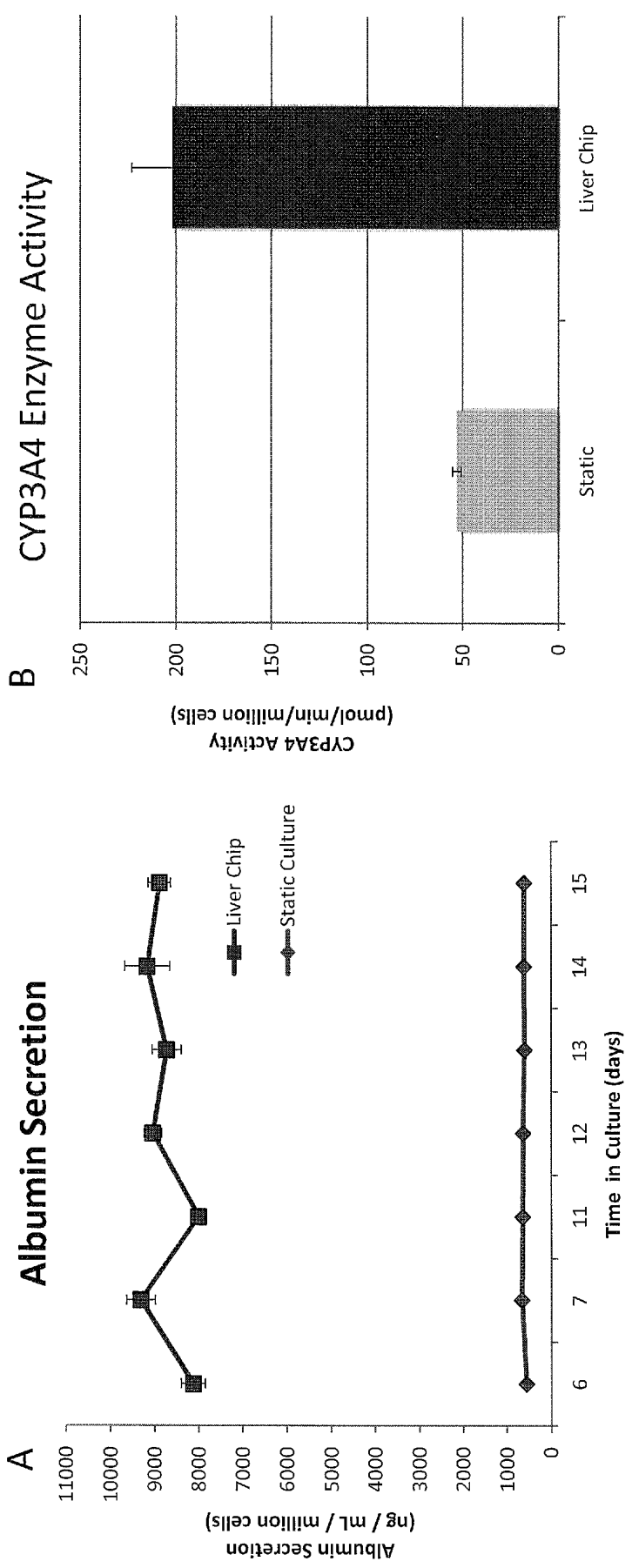
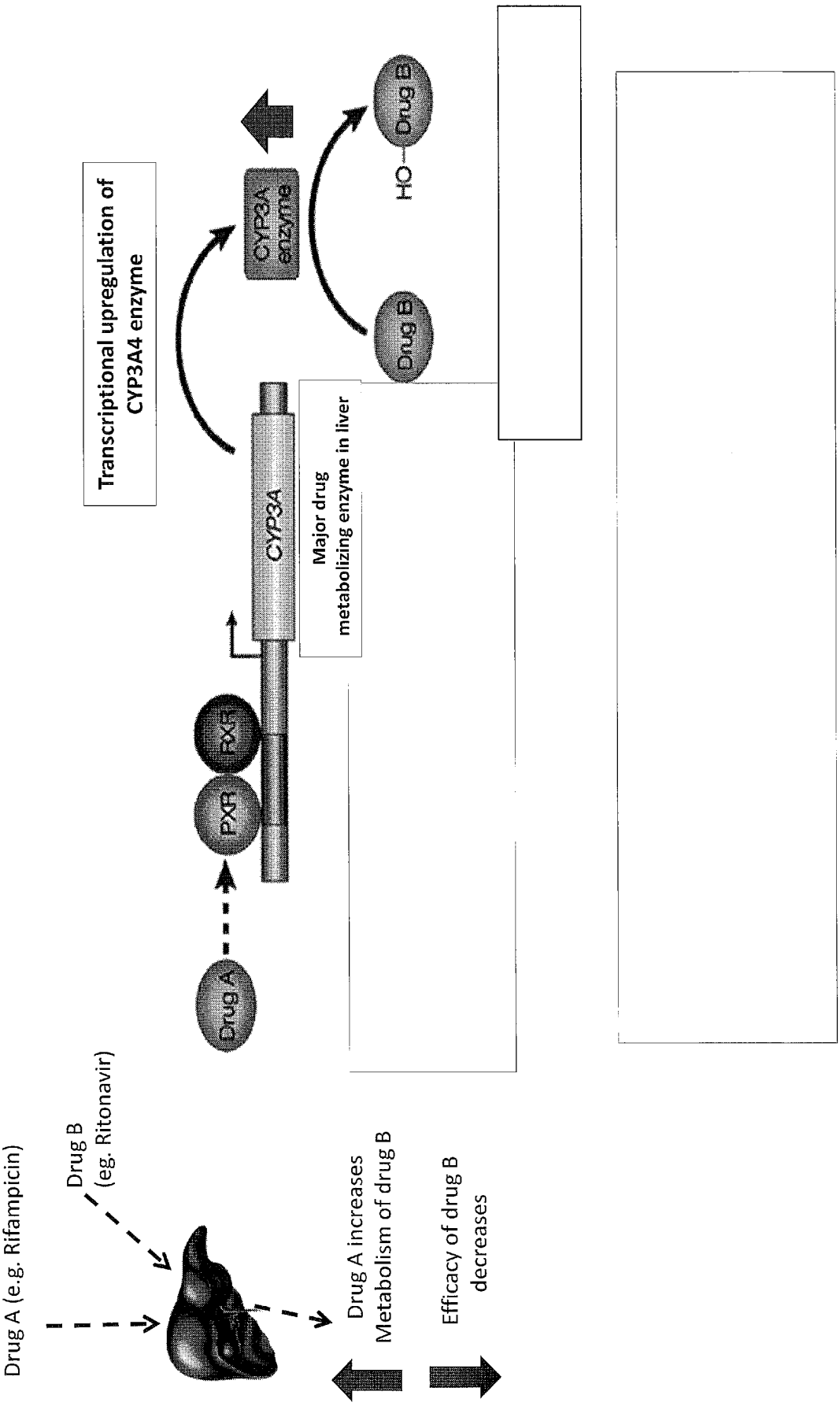
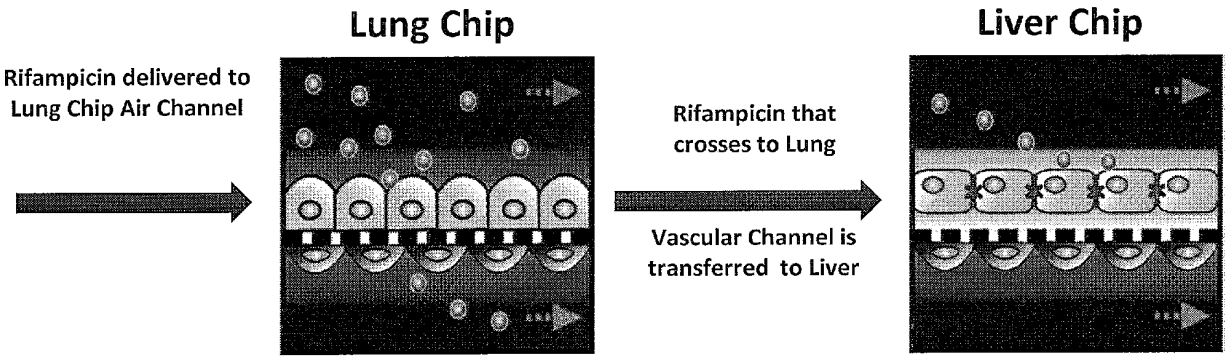

A
Albumin Secretion
Albumin Secretion (ng / mL / million cells)
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
6
7
11
12
13
14
15
Time in Culture (days)
Liver Chip
Static Culture
B
CYP3A4 Enzyme Activity
CYP3A4 Activity (pmol/min/million cells)
250
200
150
100
50
0
Static
Liver Chip

Fig. 5

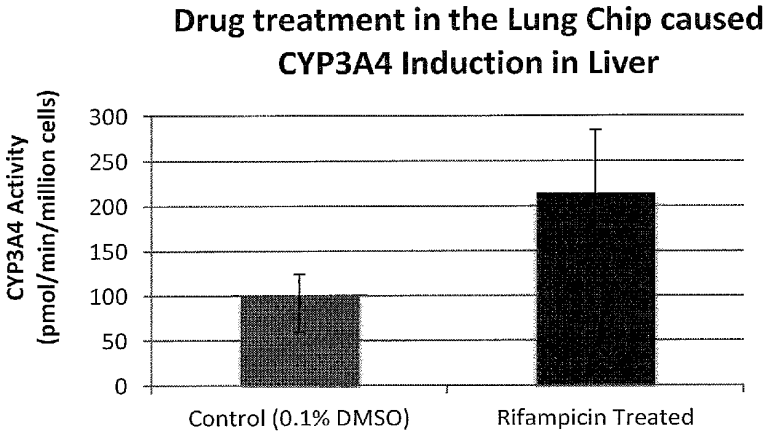

Drug A (e.g. Rifampicin)
Drug B
(eg. Ritonavir)
Drug A increases
Metabolism of drug B
Efficacy of drug B
decreases
Drug A
PXR
RXR
CYP3A
Major drug
metabolizing enzyme in liver
Transcriptional upregulation of
CYP3A4 enzyme
CYP3A
enzyme
Drug B
HO
Drug B

Figs. 6A-6B

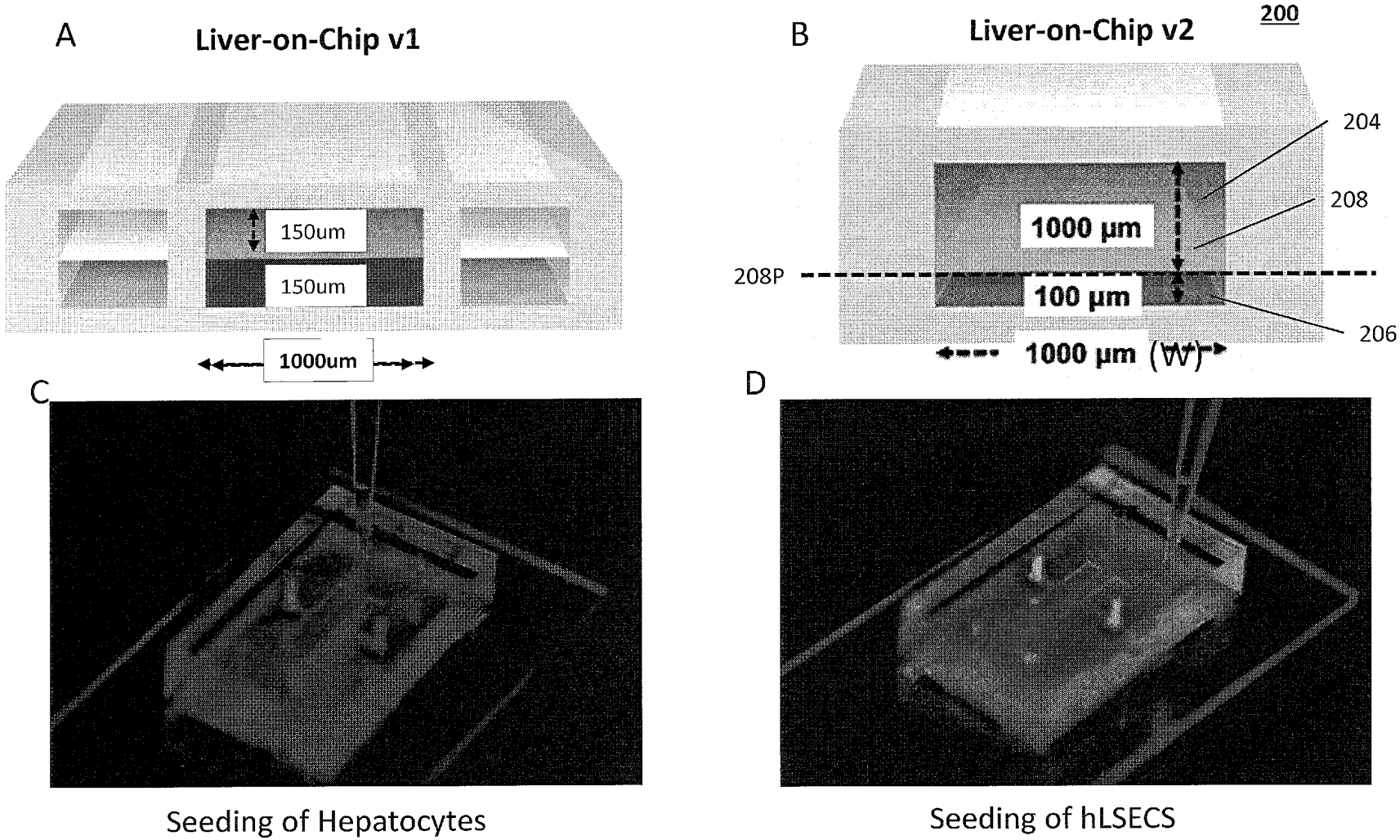
A


Lung Chip
Liver Chip
Rifampicin delivered to Lung Chip Air Channel
Rifampicin that crosses to Lung
Vascular Channel is transferred to Liver

B


Drug treatment in the Lung Chip caused CYP3A4 Induction in Liver
CYP3A4 Activity (pmol/min/million cells)
300
250
200
150
100
50
0
Control (0.1% DMSO)
Rifampicin Treated

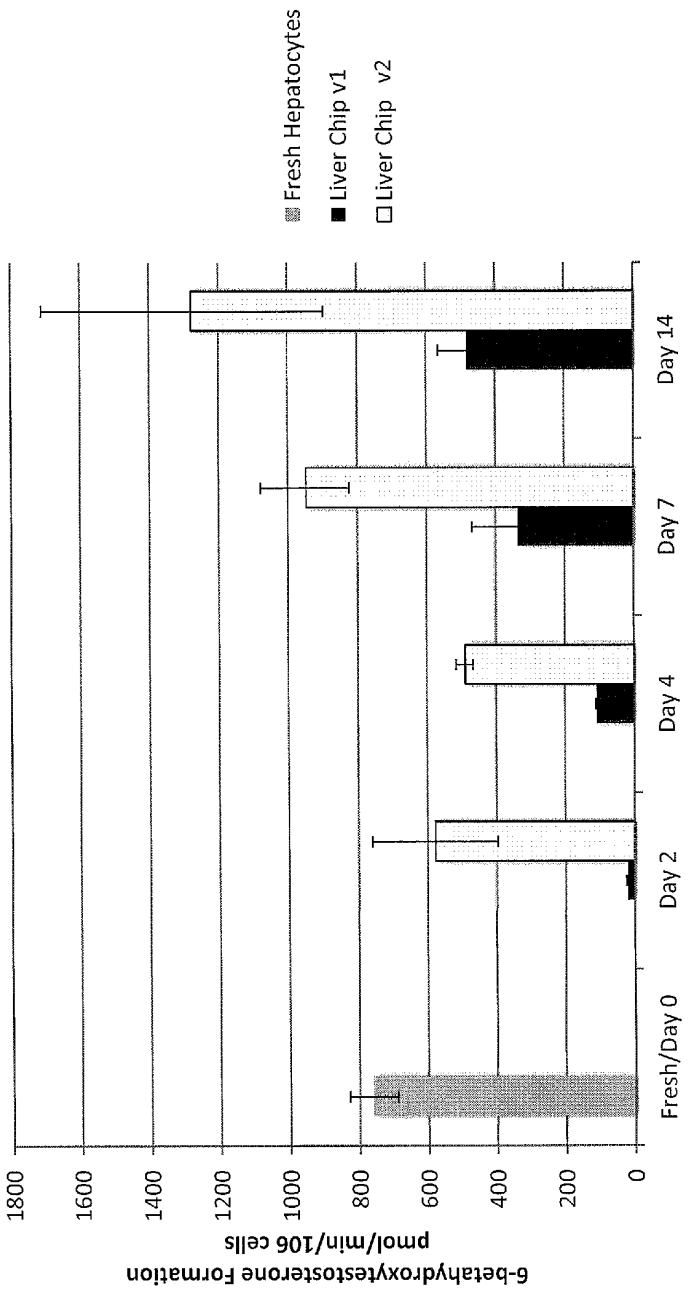
Figs. 7A-7D



Seeding of Hepatocytes

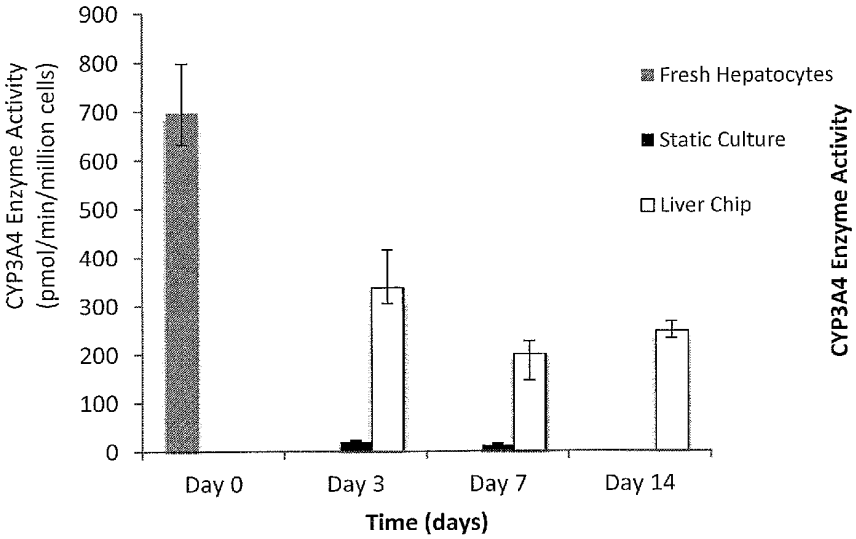
Seeding of hLSECS

Fig. 8


CYP 3A4 Activity For Human Lot8150
6-betahydroxytestosterone Formation
pmol/min/106 cells
1800
1600
1400
1200
1000
800
600
400
200
0
Fresh/Day 0
Day 2
Day 4
Day 7
Day 14
Fresh Hepatocytes
Liver Chip v1
Liver Chip v2

Figs. 9A-9B

A


CYP3A4 Activity for Donor 1
CYP3A4 Enzyme Activity (pmol/min/million cells)
900
800
700
600
500
400
300
200
100
0
Day 0
Day 3
Day 7
Day 14
Time (days)
Fresh Hepatocytes
Static Culture
Liver Chip

B

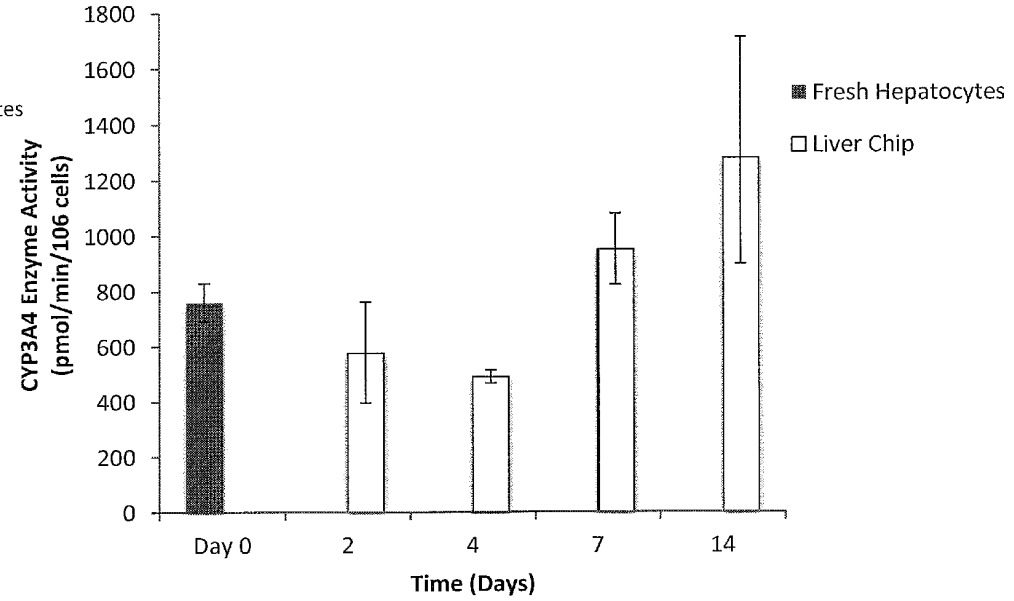

CYP 3A4 Activity For Human Donor 2
CYP3A4 Enzyme Activity (pmol/min/106 cells)
1800
1600
1400
1200
1000
800
600
400
200
0
Day 0
2
4
7
14
Time (Days)
Fresh Hepatocytes
Liver Chip

Fig. 10

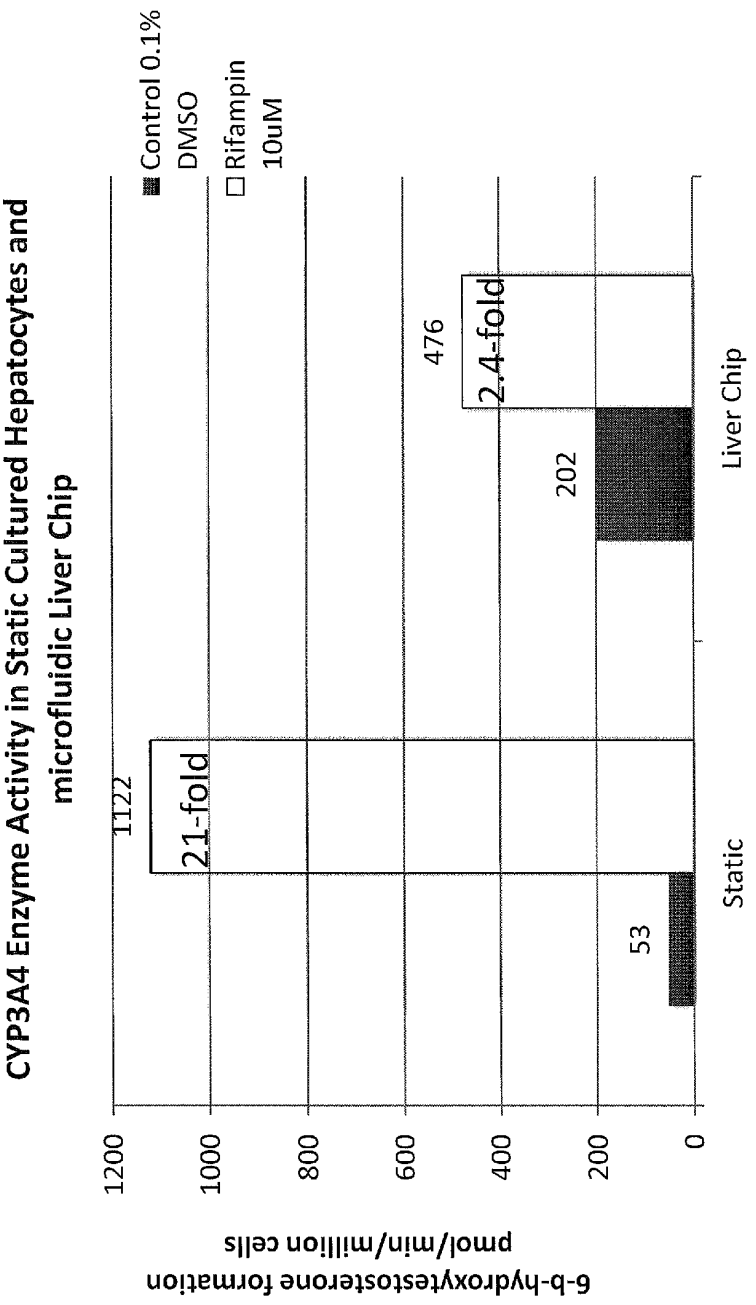
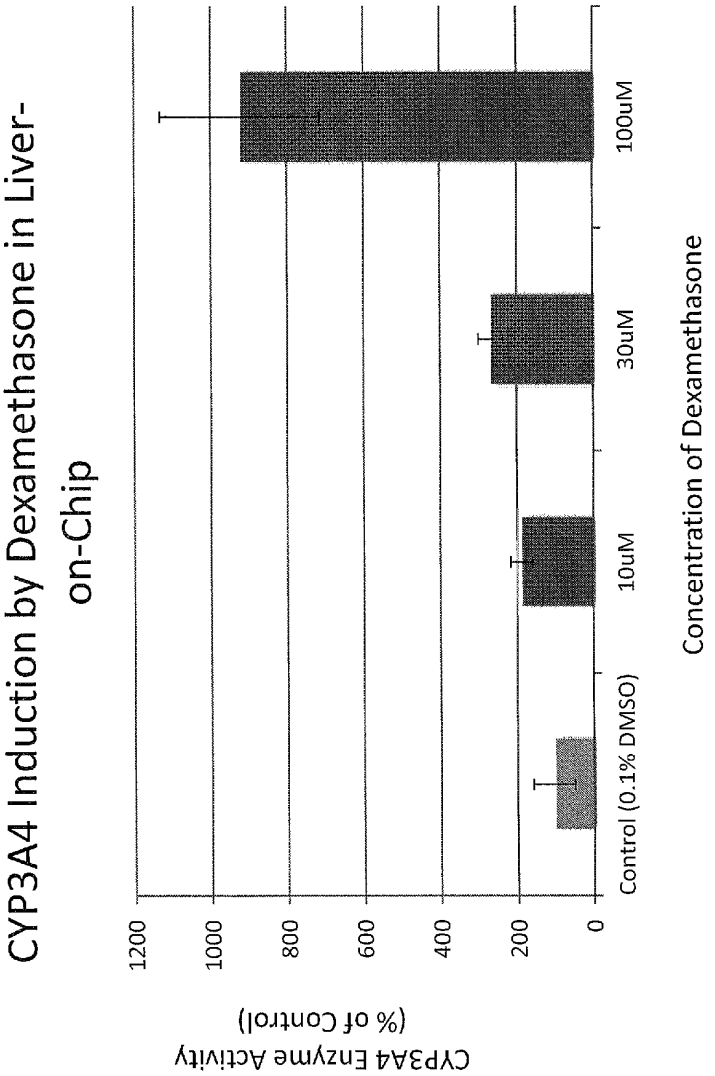
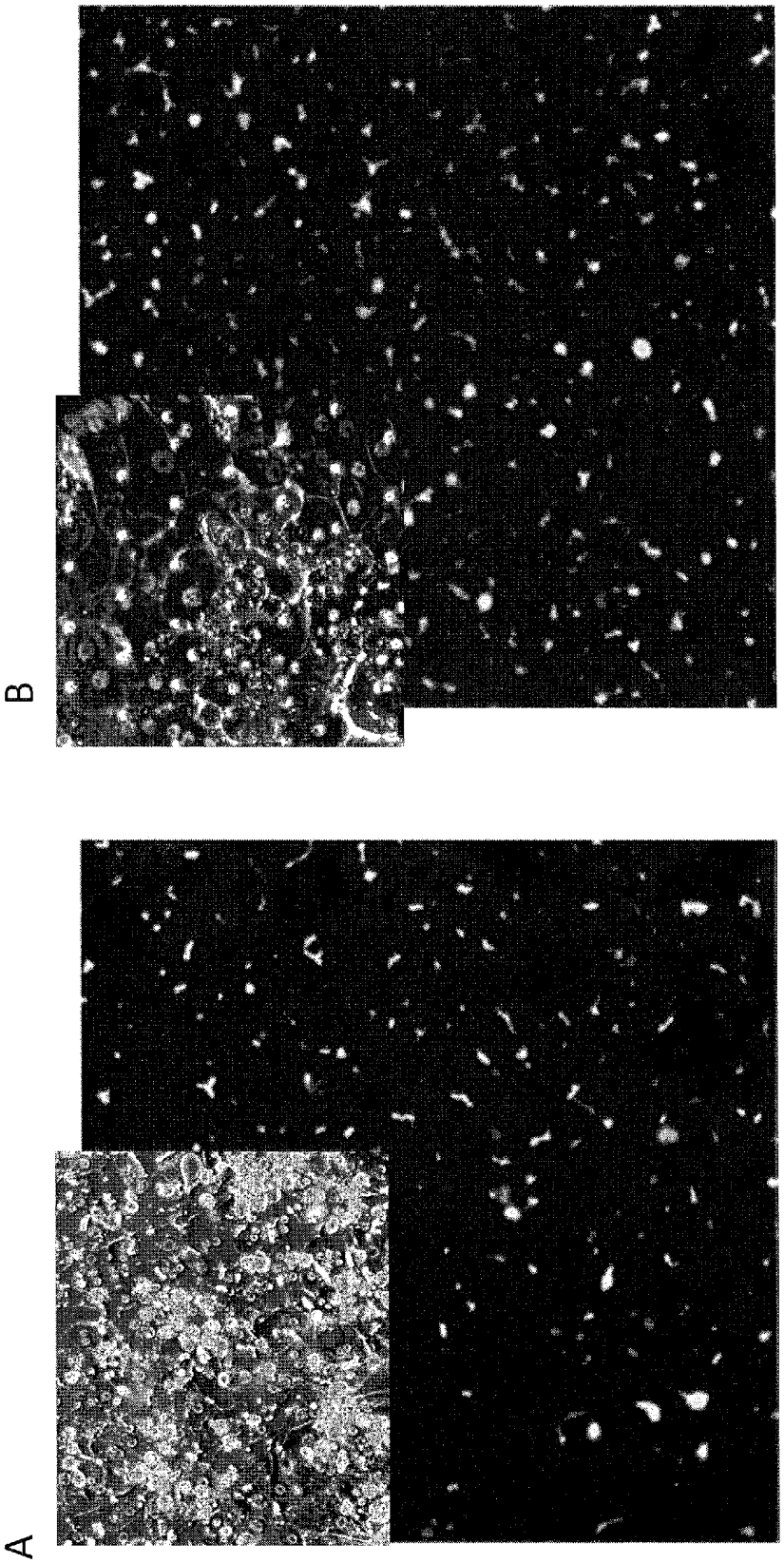

CYP3A4 Enzyme Activity in Static Cultured Hepatocytes and microfluidic Liver Chip
Control 0.1% DMSO
Rifampin 10uM
6-b-hydroxytestosterone formation pmol/min/million cells
1200
1000
800
600
400
200
0
1122
21-fold
53
476
2.4-fold
202
Static
Liver Chip

Fig. 11


CYP3A4 Induction by Dexamethasone in Liver-on-Chip
CYP3A4 Enzyme Activity (% of Control)
1200
1000
800
600
400
200
0
Control (0.1% DMSO)
10uM
30uM
100uM
Concentration of Dexamethasone

Figs. 12A-12B



Figs. 13A-13B

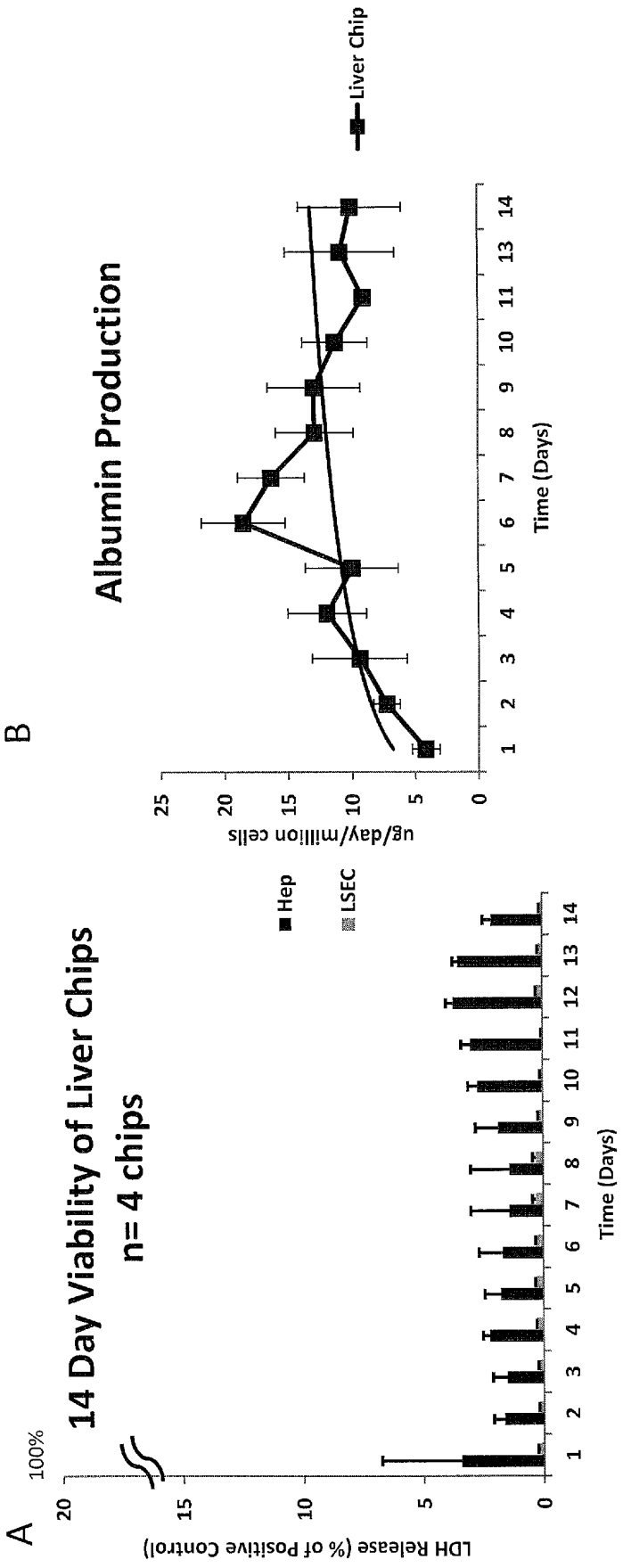
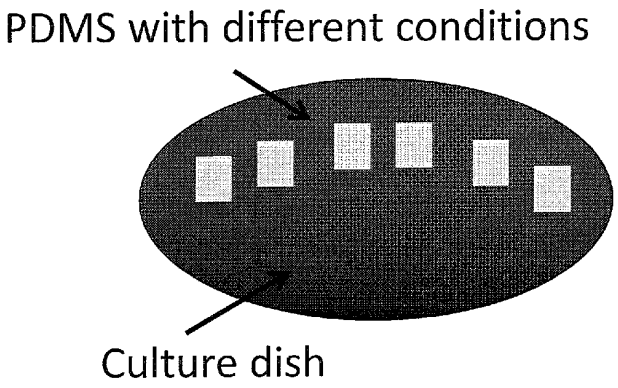

A
14 Day Viability of Liver Chips
n= 4 chips
100%
LDH Release (% of Positive Control)
Time (Days)
Hep
LSEC
B
Albumin Production
ug/day/million cells
Time (Days)
Liver Chip

Fig. 14A



1 PDMS Surface treatment

- Plasma
- APTES

2 ECM Coating

ECM	Concentration (mg/ml)
Collagen I	1, 0.5, 0.5+Matrigel 0.1
Fibronectin	1, 0.5, 0.5 +Matrigel 0.1
Collagen IV	1
Mixture	Col I+Col IV+FN

3 ECM Overlay

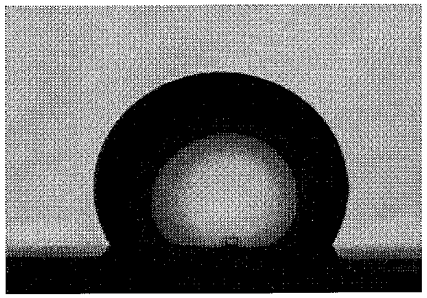
- Matrigel *vs* Mixture of Matrigel+ Fibronectin

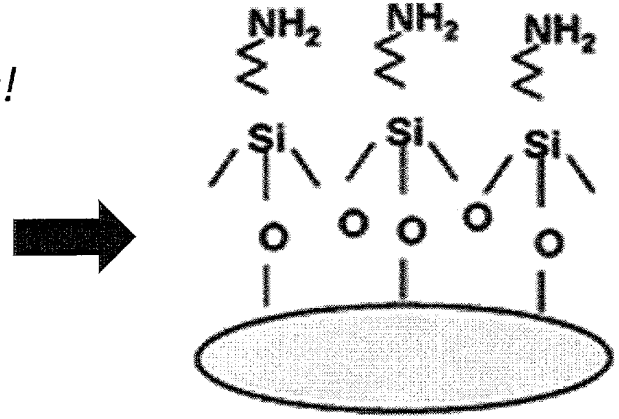
4 Medium

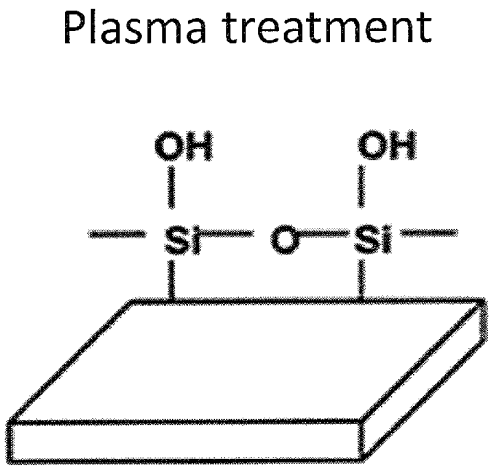
Basal Medium	Supplement
WEM complete (William's E medium + L-Glutamax, ITS +, Dexamethasone, Penn/Strep)	10% FBS
	SBTI (Soybean Trypsin Inhibitor) 1mg/ml
	EGF (Epidermal growth factor) 200ng/ml

Fig. 14B
PDMS Surface Treatment

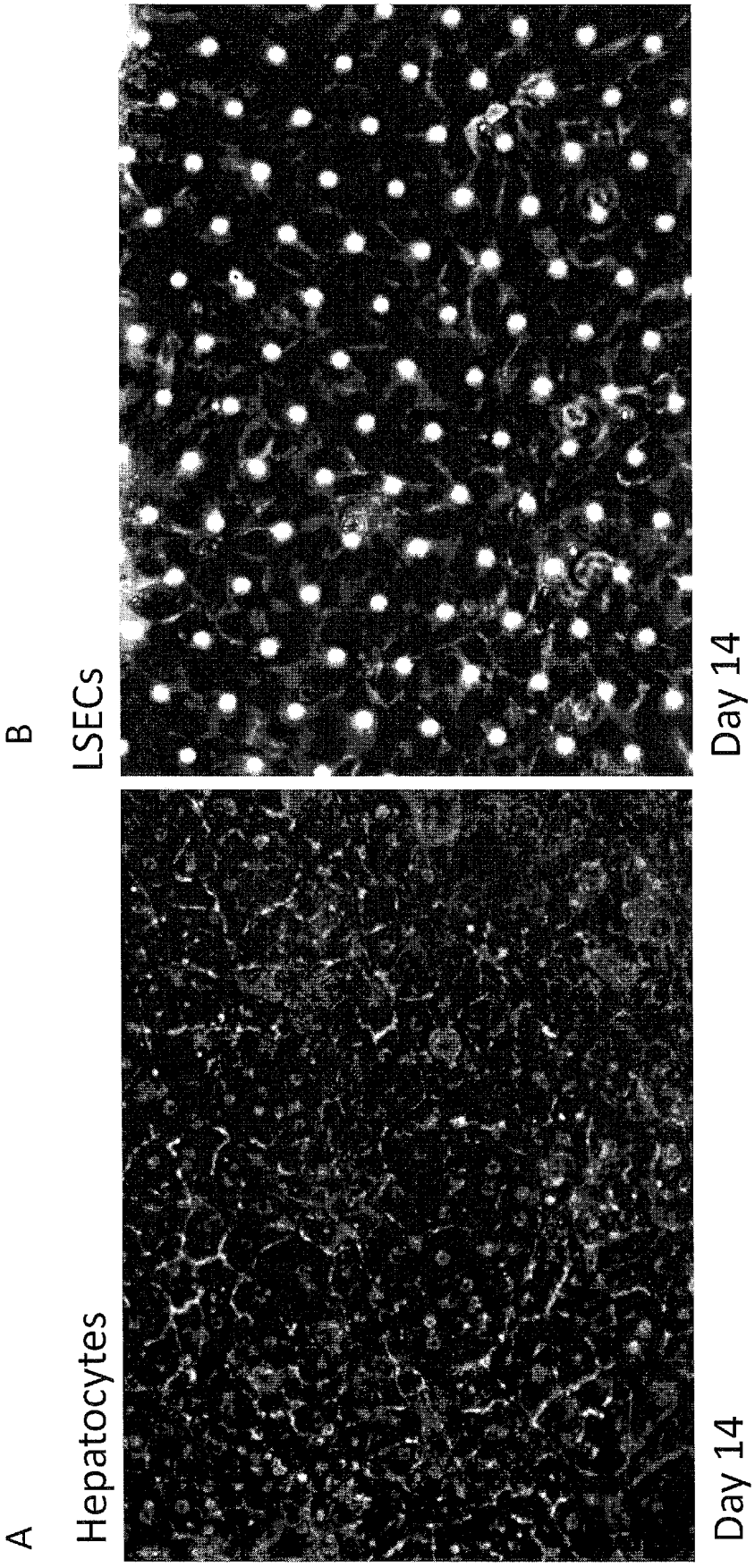
PDMS is hydrophobic!



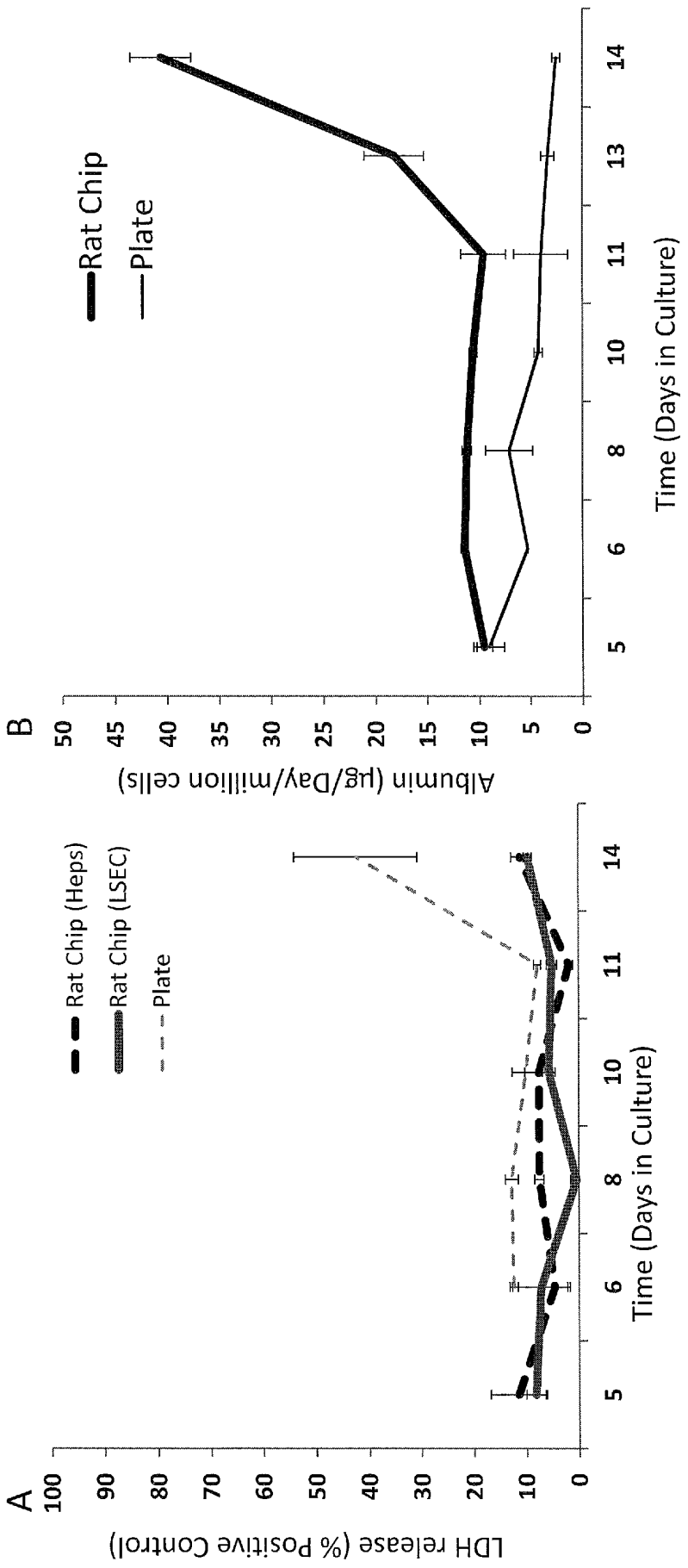




Figs. 15A-15B



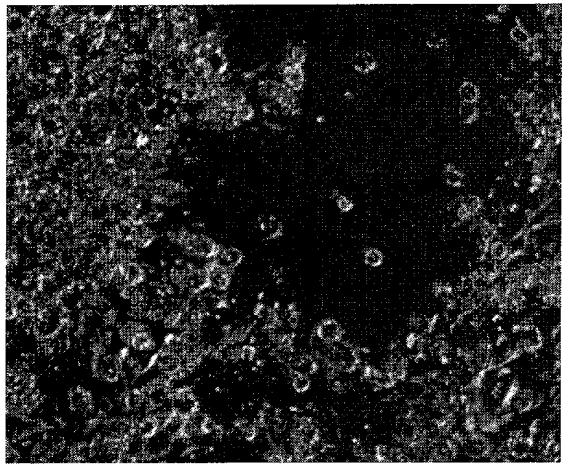
Figs. 16A-16B


A
LDH release (% Positive Control)
100
90
80
70
60
50
40
30
20
10
0
5
6
8
10
11
14
Time (Days in Culture)
Rat Chip (Heps)
Rat Chip (LSEC)
Plate
B
Albumin (µg/Day/million cells)
50
45
40
35
30
25
20
15
10
5
0
5
6
8
10
11
13
14
Time (Days in Culture)
Rat Chip
Plate

Figs. 17A-17C

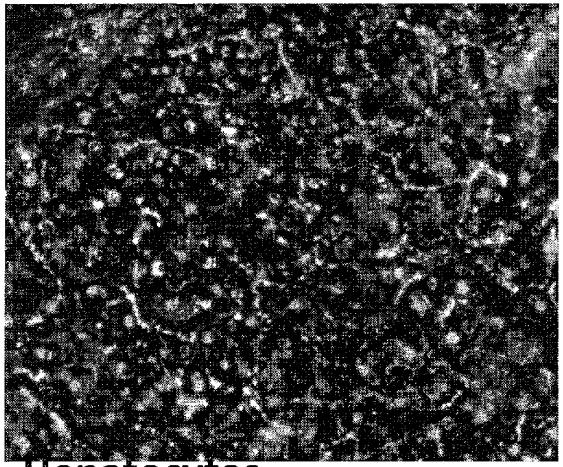
A

Static Culture –Plate Day 28

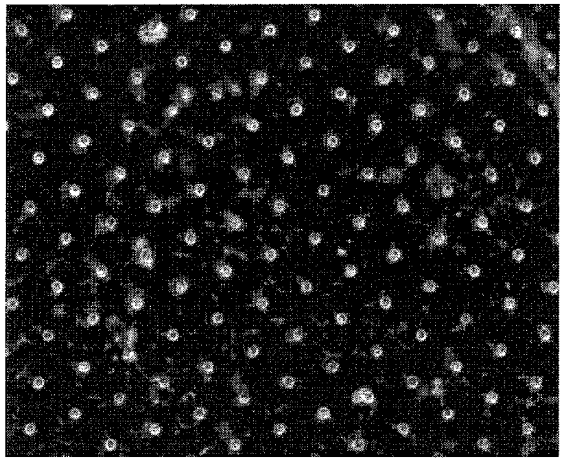


B

Rat Liver Chip –Day 28

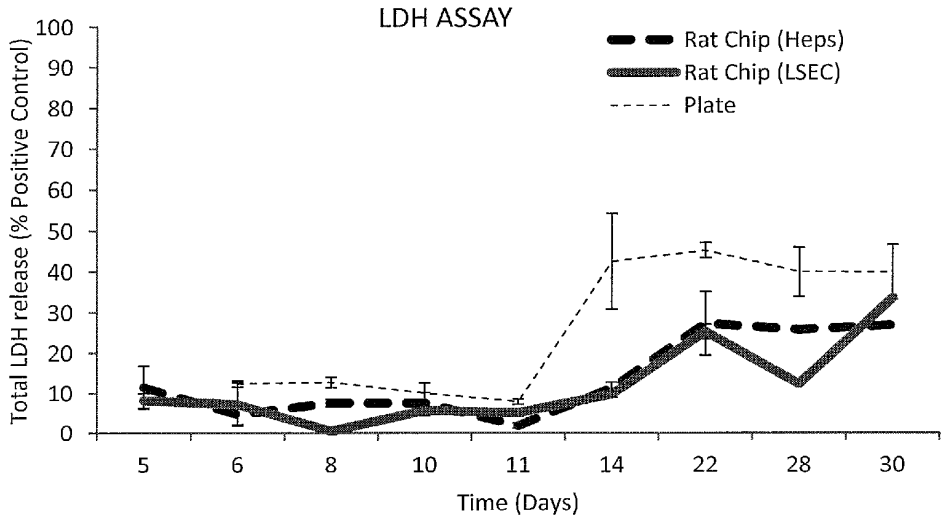


Hepatocytes

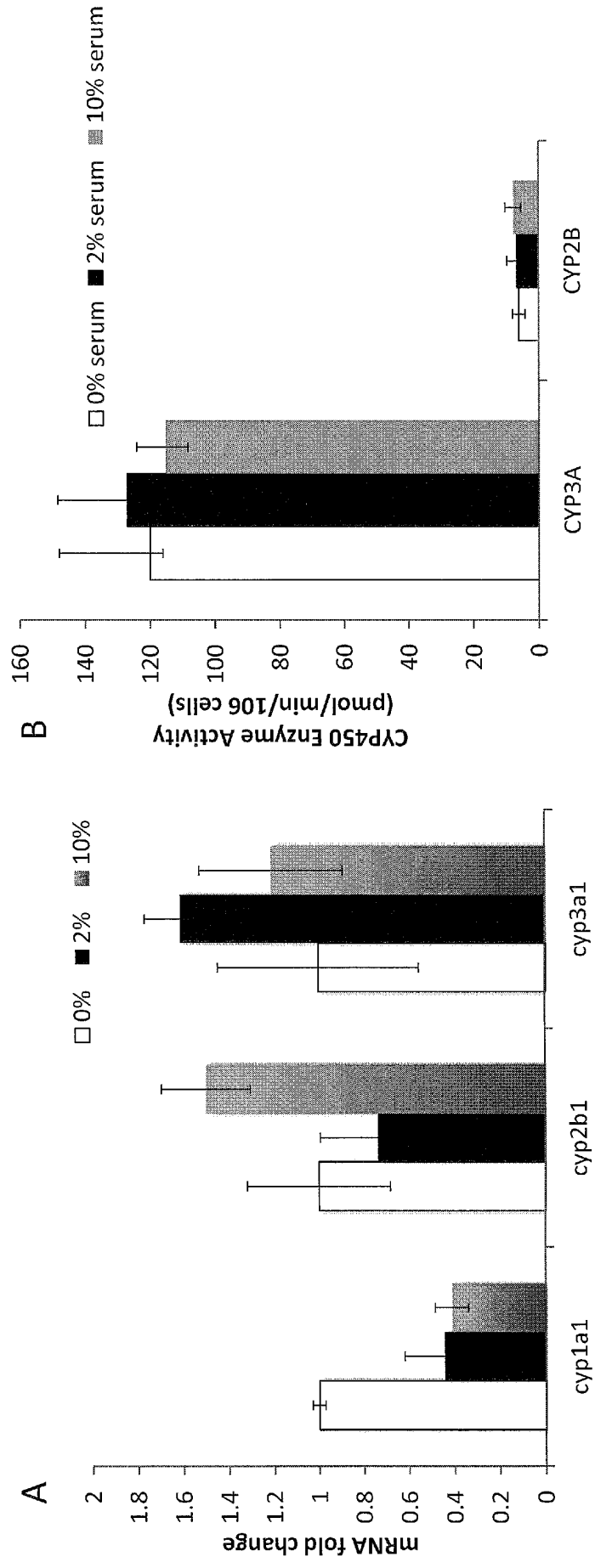


rLSECs

C



Figs. 18A-18B


Rat Hepatocytes in Conventional Sandwich Plate Culture (Day 7)
A
mRNA fold change
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
□0% ■2% ■10%
cyp1a1
cyp2b1
cyp3a1
B
CYP450 Enzyme Activity (pmol/min/10^6 cells)
0
20
40
60
80
100
120
140
160
□0% serum ■2% serum ■10% serum
CYP3A
CYP2B

Figs. 19A-19C

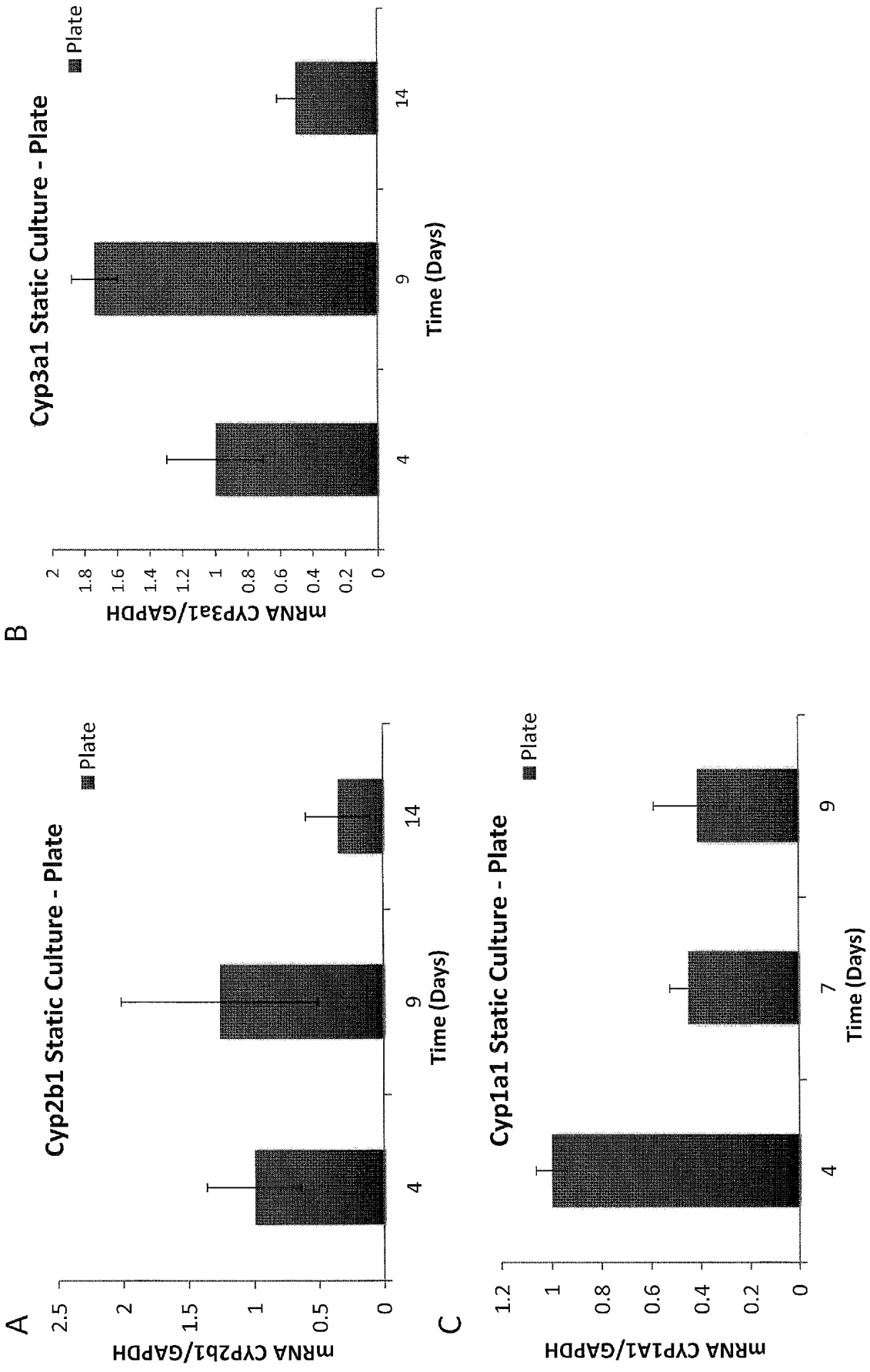

A
Cyp2b1 Static Culture - Plate
Plate
mRNA CYP2b1/GAPDH
2.5
2
1.5
1
0.5
0
4
9
14
Time (Days)
B
Cyp3a1 Static Culture - Plate
Plate
mRNA CYP3a1/GAPDH
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
4
9
14
Time (Days)
C
Cyp1a1 Static Culture - Plate
Plate
mRNA CYP1A1/GAPDH
1.2
1
0.8
0.6
0.4
0.2
0
4
7
9
Time (Days)

Fig. 20A

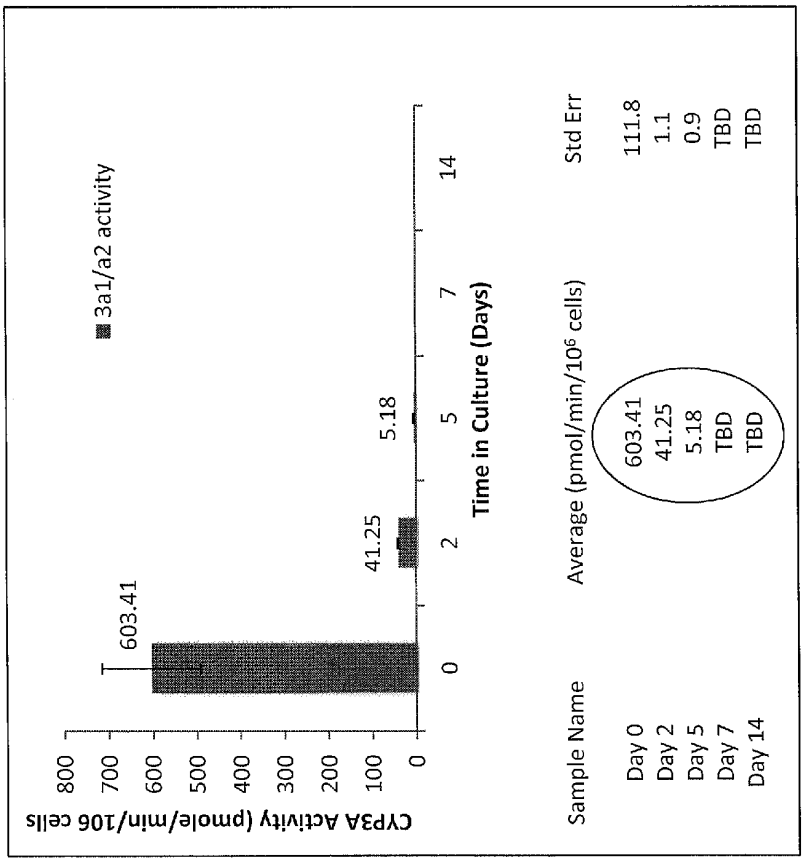
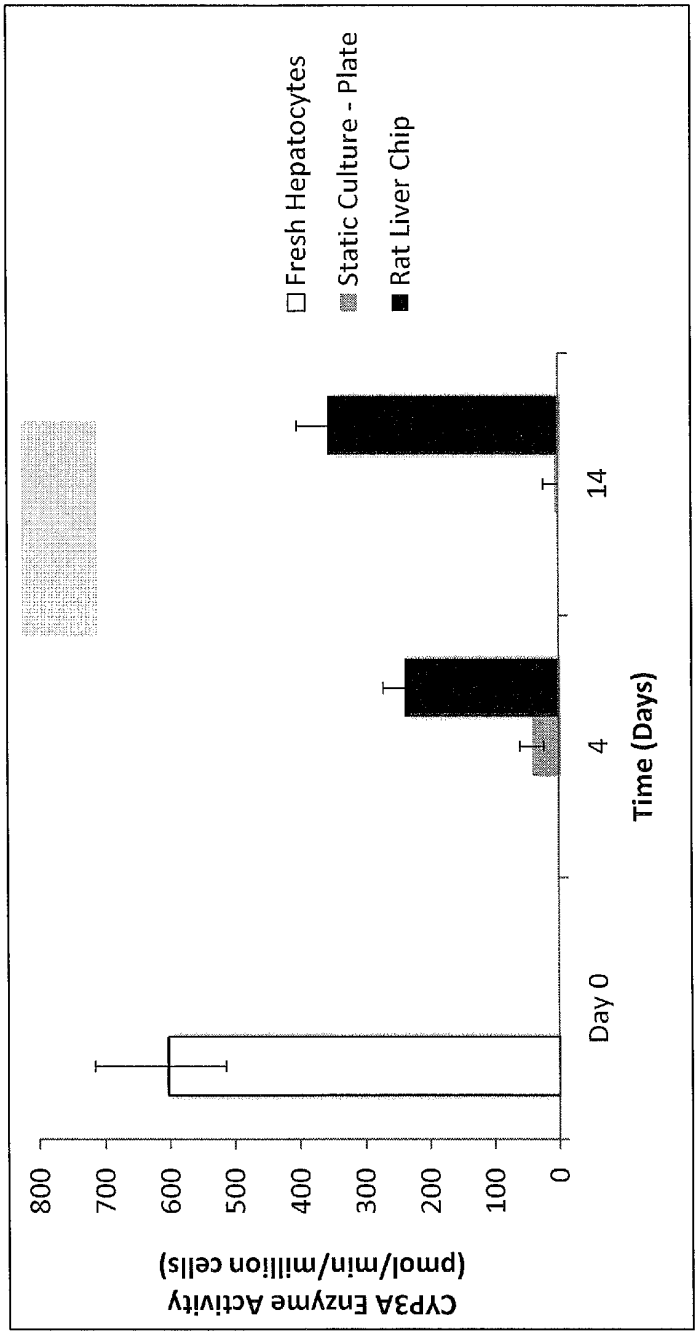

CYP3A Activity (pmole/min/106 cells
800
700
600
500
400
300
200
100
0
3a1/a2 activity
603.41
41.25
5.18
0
2
5
7
14
Time in Culture (Days)
Sample Name
Average (pmol/min/10^6 cells)
Std Err
Day 0
603.41
111.8
Day 2
41.25
1.1
Day 5
5.18
0.9
Day 7
TBD
TBD
Day 14
TBD
TBD

Fig. 20B


CYP3A Enzyme Activity
(pmol/min/million cells)
800
700
600
500
400
300
200
100
0
Day 0
4
14
Time (Days)
Fresh Hepatocytes
Static Culture - Plate
Rat Liver Chip

Figs. 21A-21C

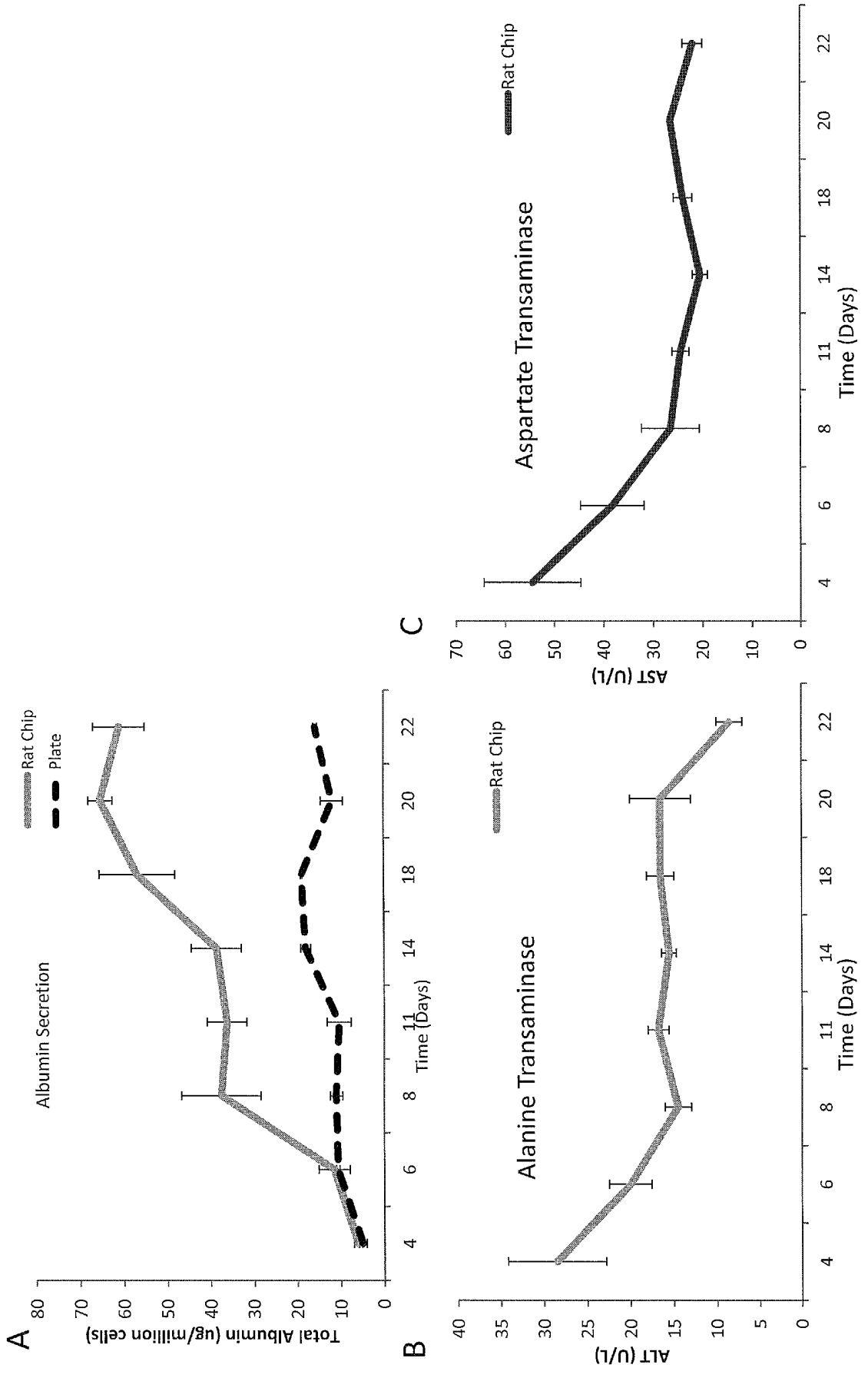
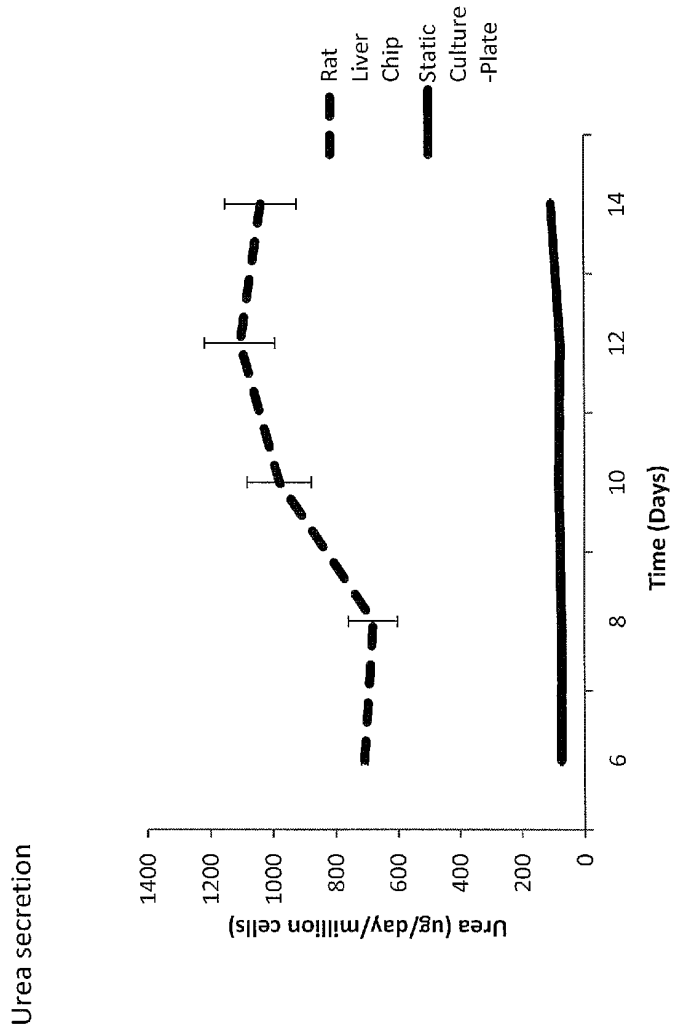
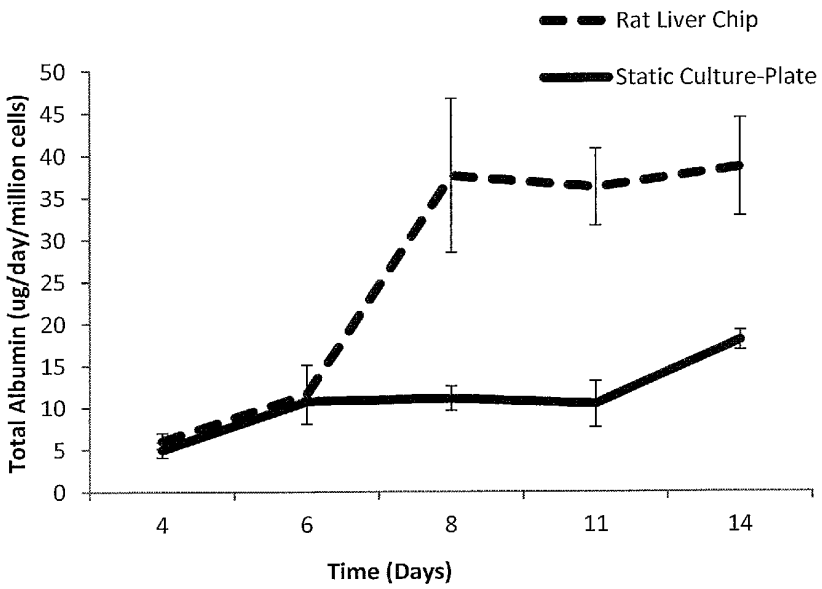

A
Albumin Secretion
Rat Chip
Plate
Total Albumin (ug/million cells)
Time (Days)
B
Alanine Transaminase
Rat Chip
ALT (U/L)
Time (Days)
C
Aspartate Transaminase
Rat Chip
AST (U/L)
Time (Days)

Fig. 22

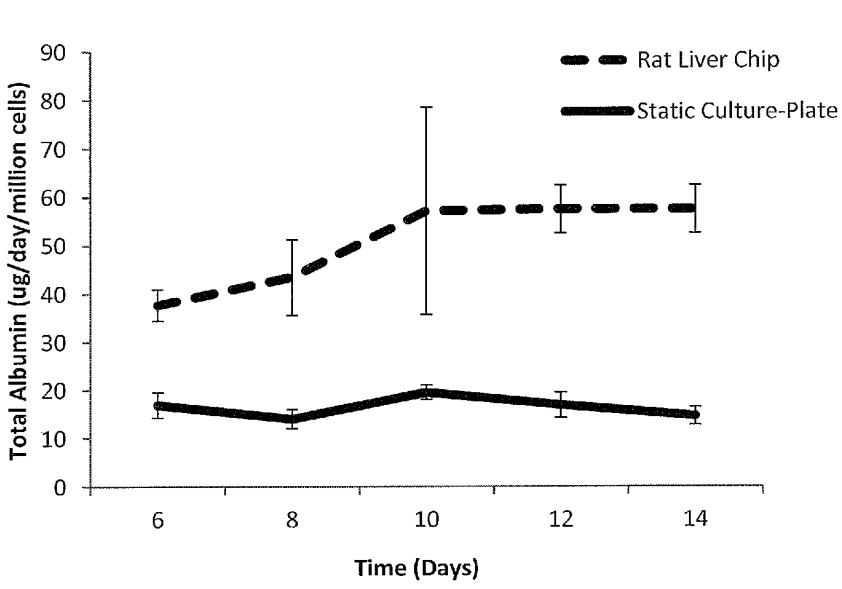

Urea secretion
Urea (ug/day/million cells)
1400
1200
1000
800
600
400
200
0
6
8
10
12
14
Time (Days)
Rat Liver Chip
Static Culture -Plate

Figs. 23A-23B

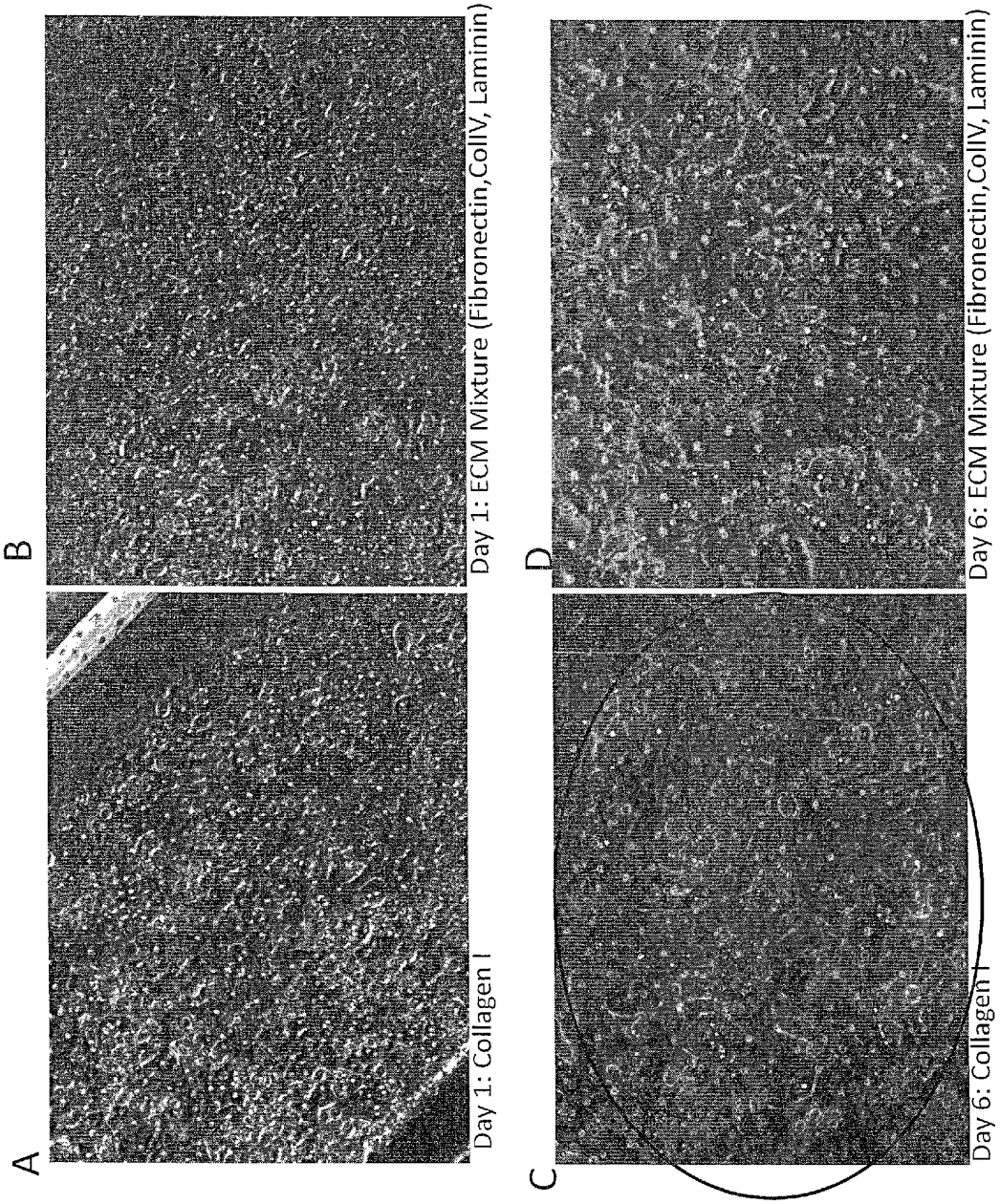
A



B



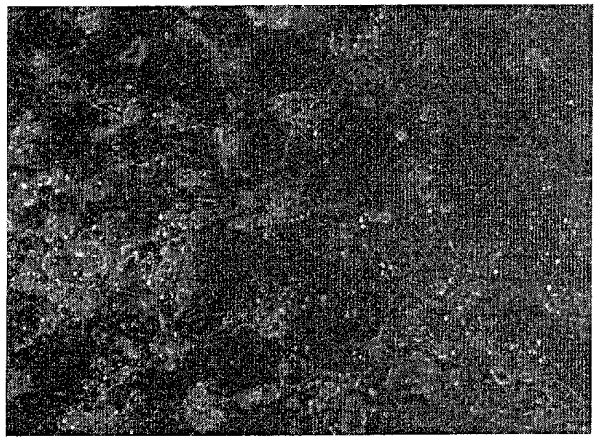
Figs. 24A-24D


A
B
Day 1: Collagen I
Day 1: ECM Mixture (Fibronectin,CollV, Laminin)
C
D
Day 6: Collagen I
Day 6: ECM Mixture (Fibronectin,CollV, Laminin)

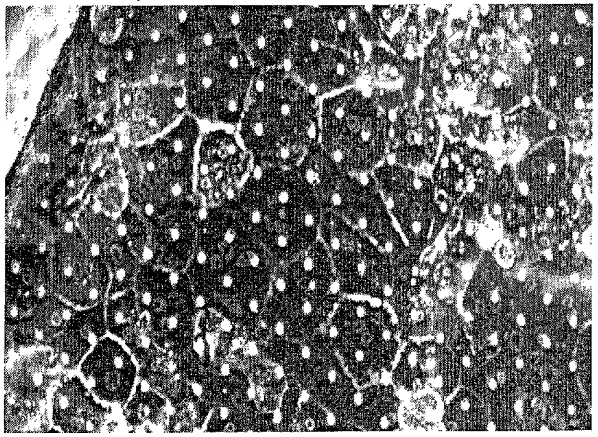
Figs. 25A-25C

A

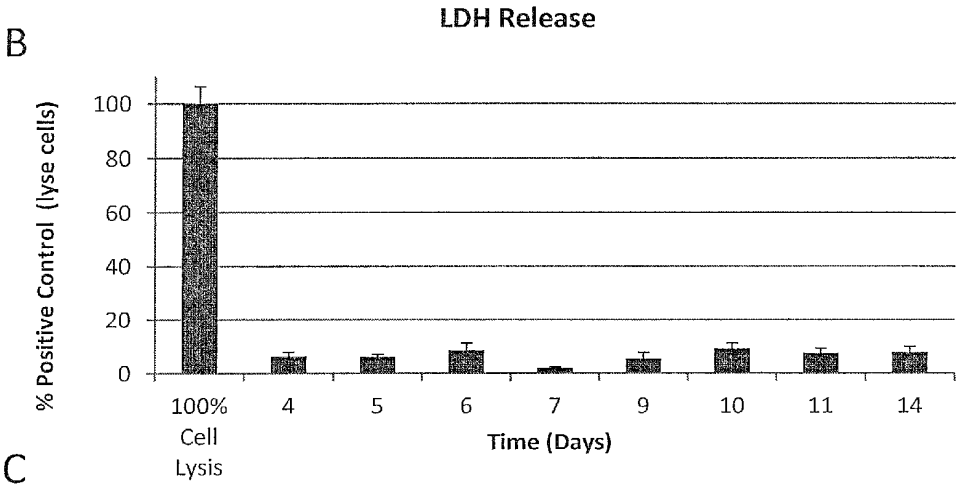
Static Culture – Plate D14



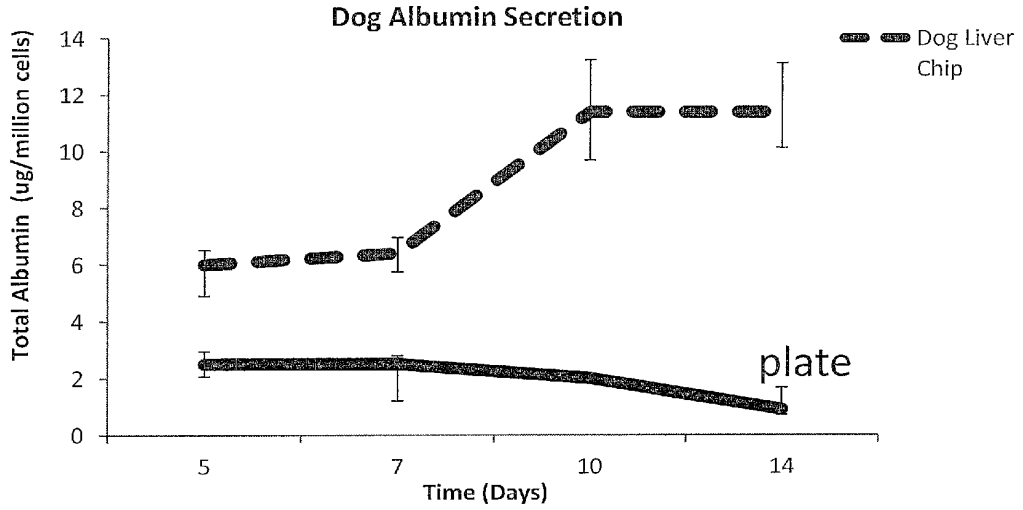
Liver Chip D10



B



C



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Figs. 26A-26B

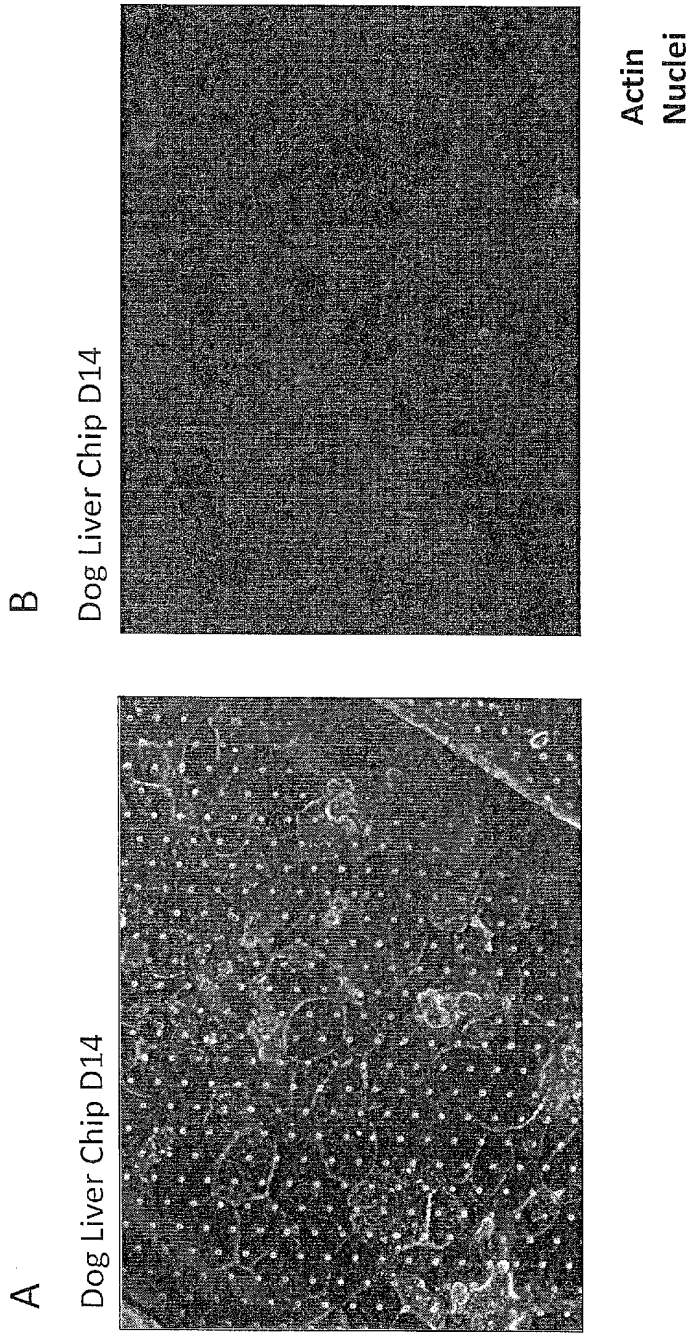


FIG. 27B

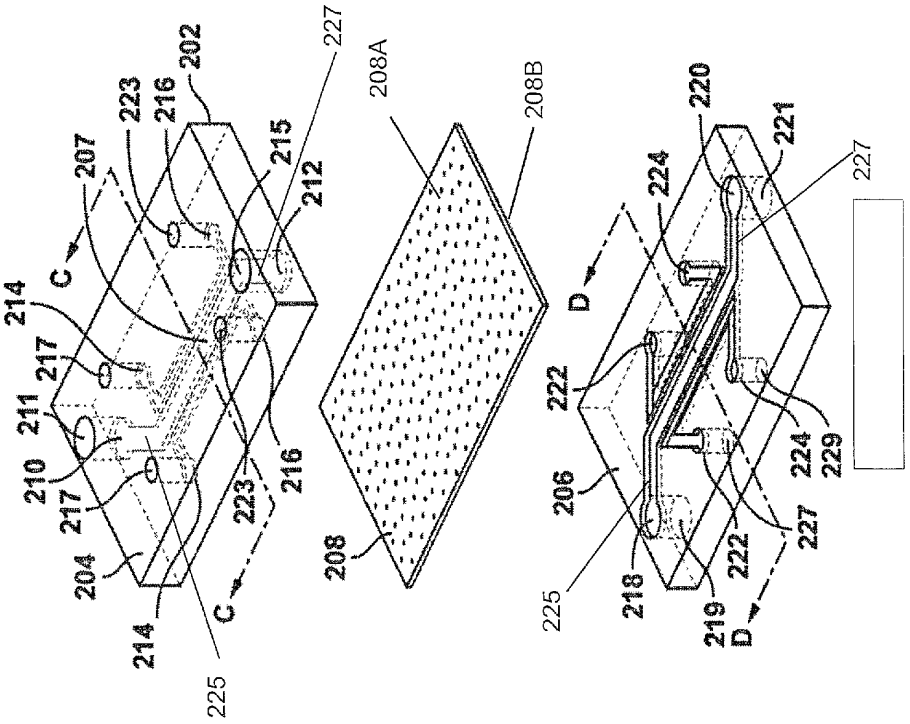


FIG. 27A

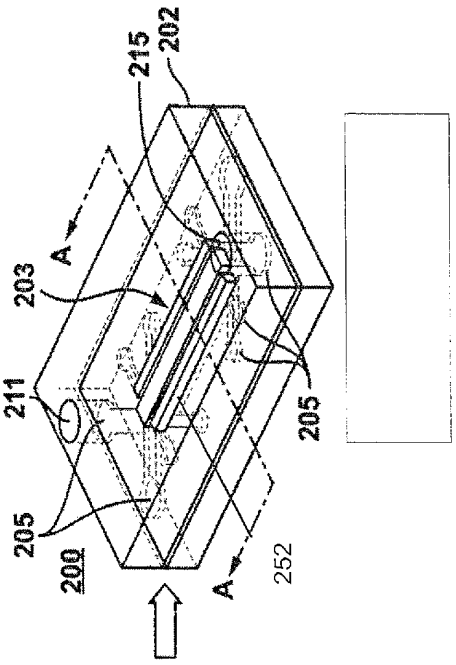


FIG. 28

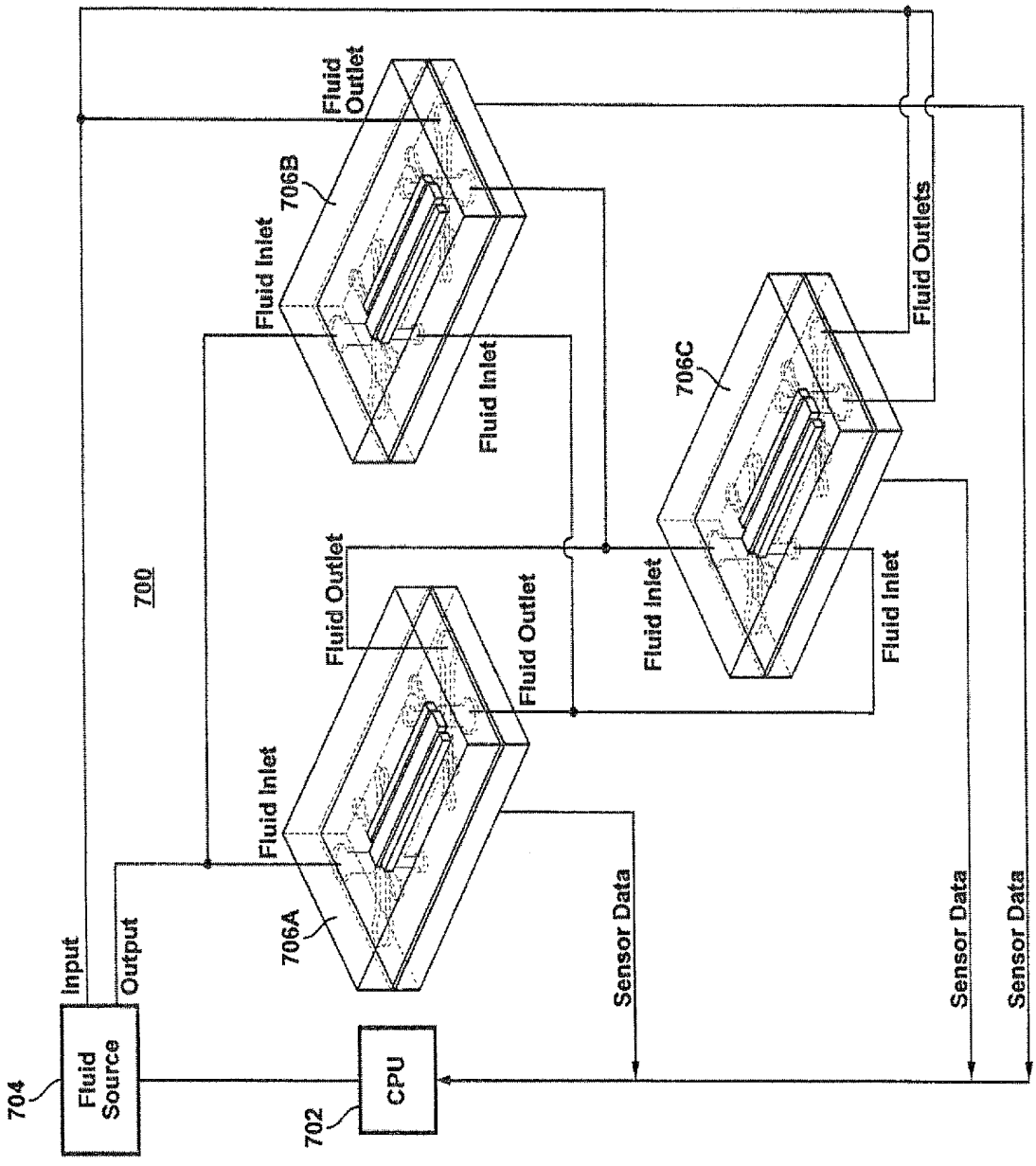
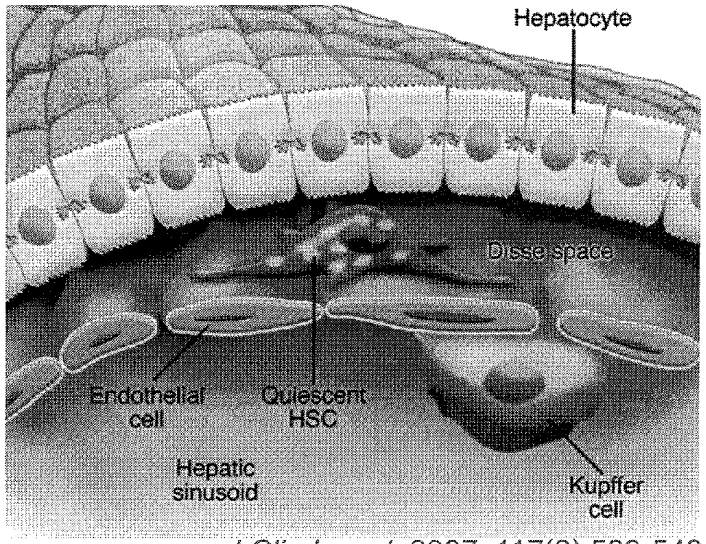

704
Fluid Source
Input
Output
700
702
CPU
706A
706B
706C
Fluid Inlet
Fluid Outlet
Fluid Outlets
Sensor Data

FIG. 29

In vivo

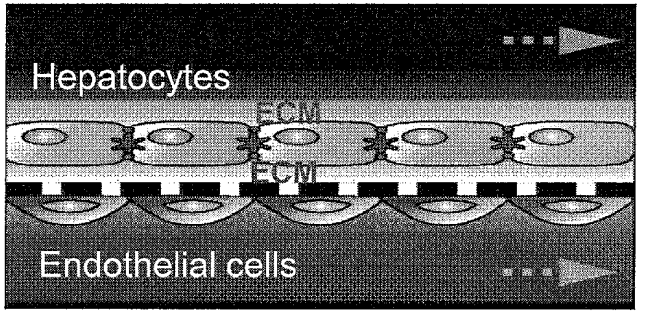


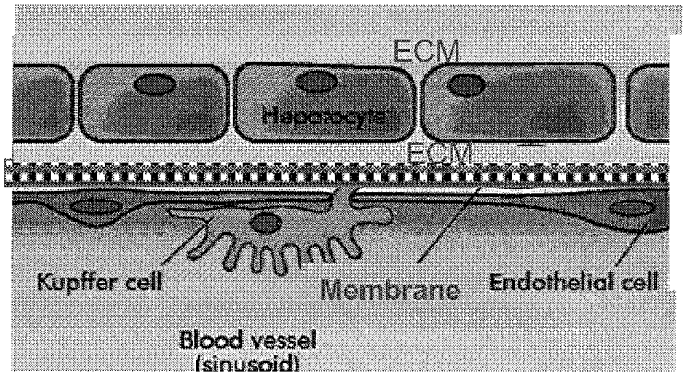
J Clin Invest. 2007; 117(3):539-548.

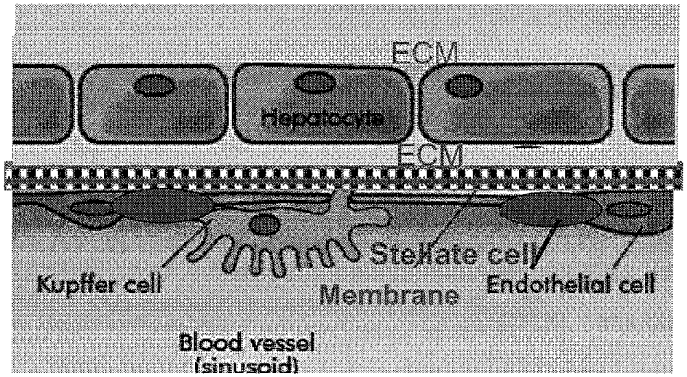
Co-culture
(Hepatocyte + LSEC)

Tri-culture
(Hepatocyte + LSEC + Kupffer cell)

Quadruple-culture
(Hepatocyte + LSEC + Kupffer cell + Stellate cell)

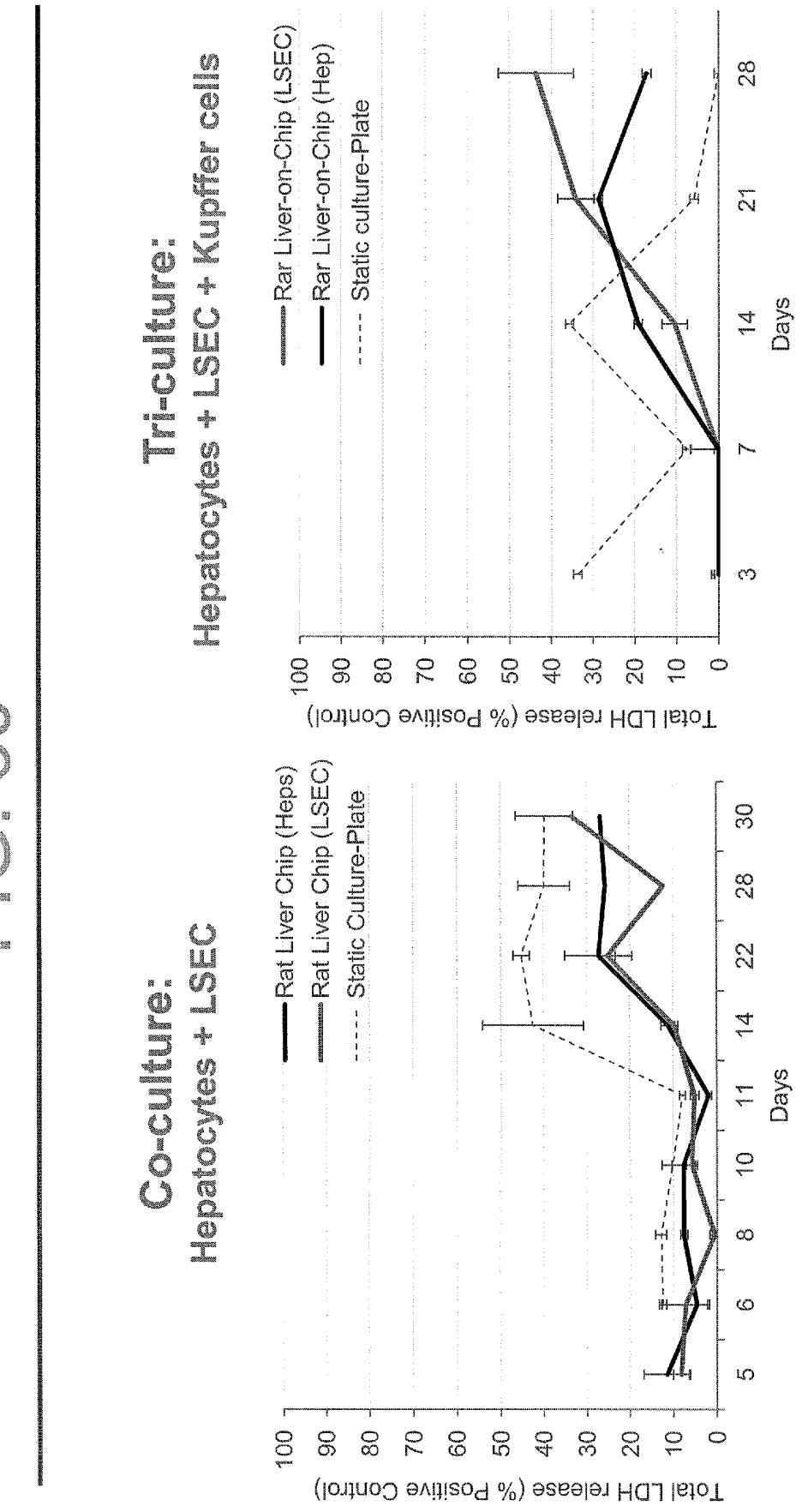






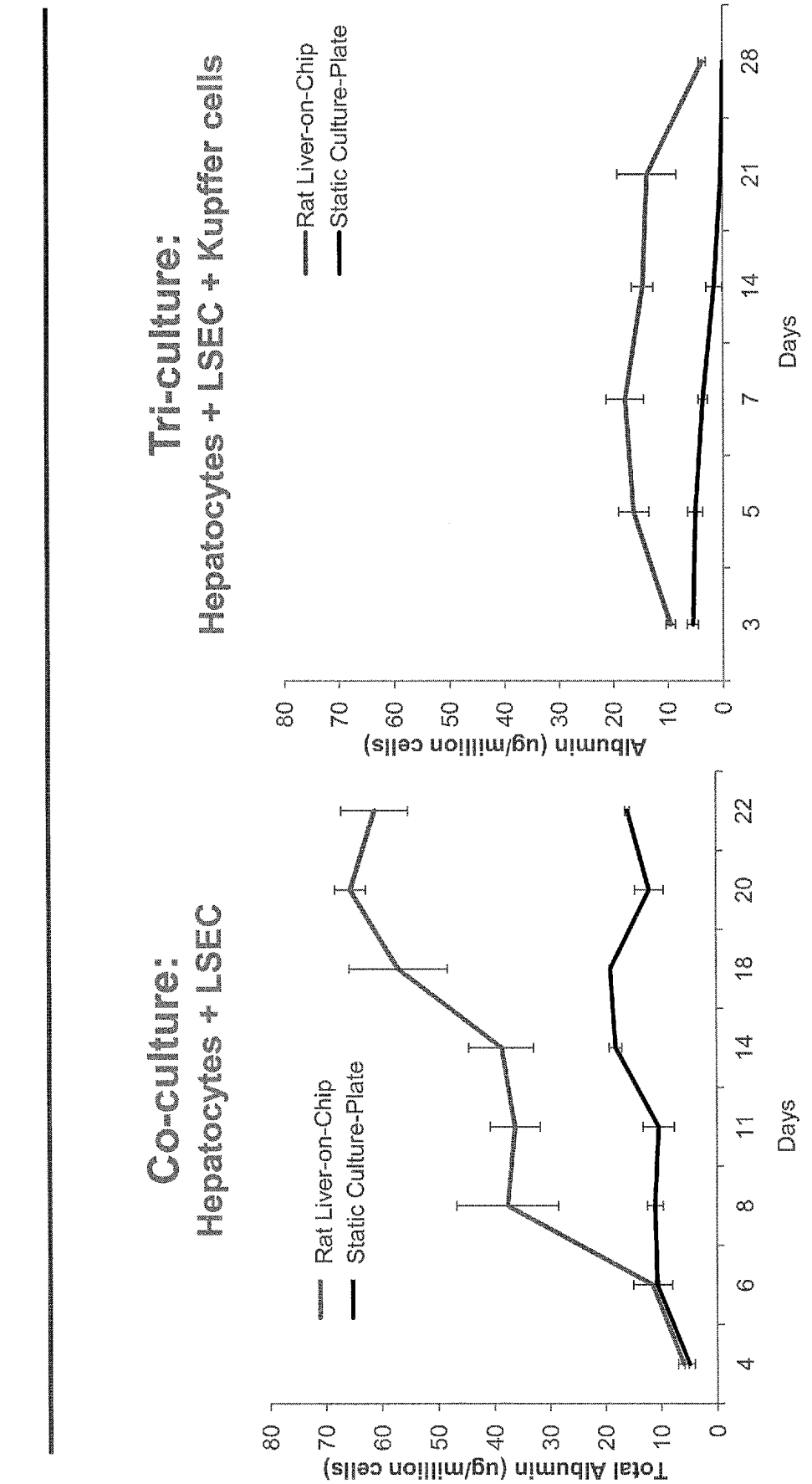
emulate

FIG. 30



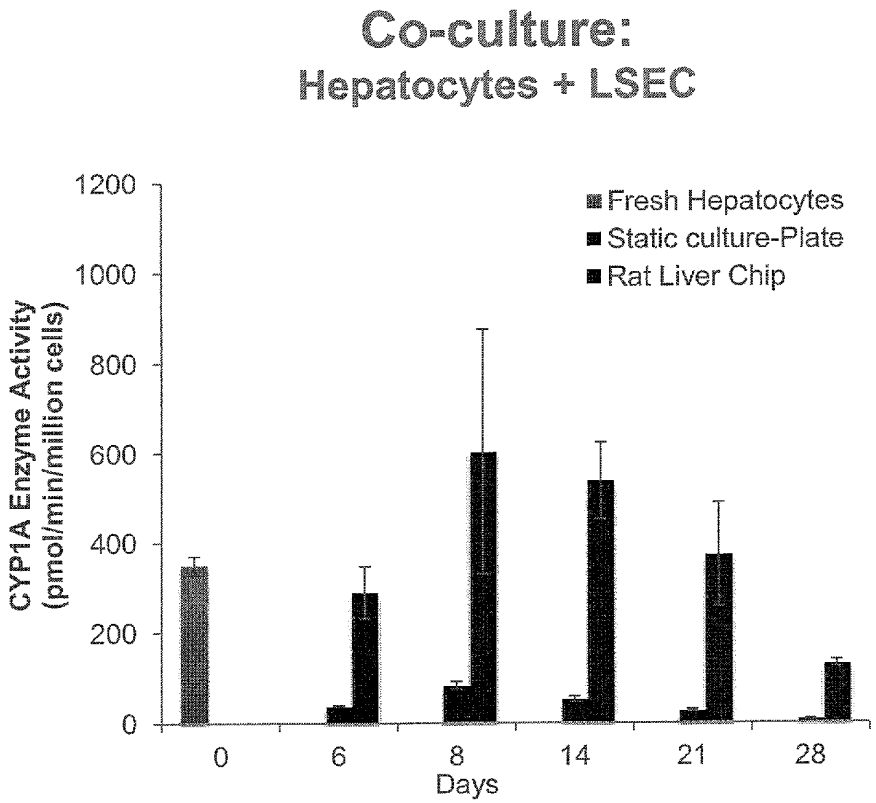
emulate

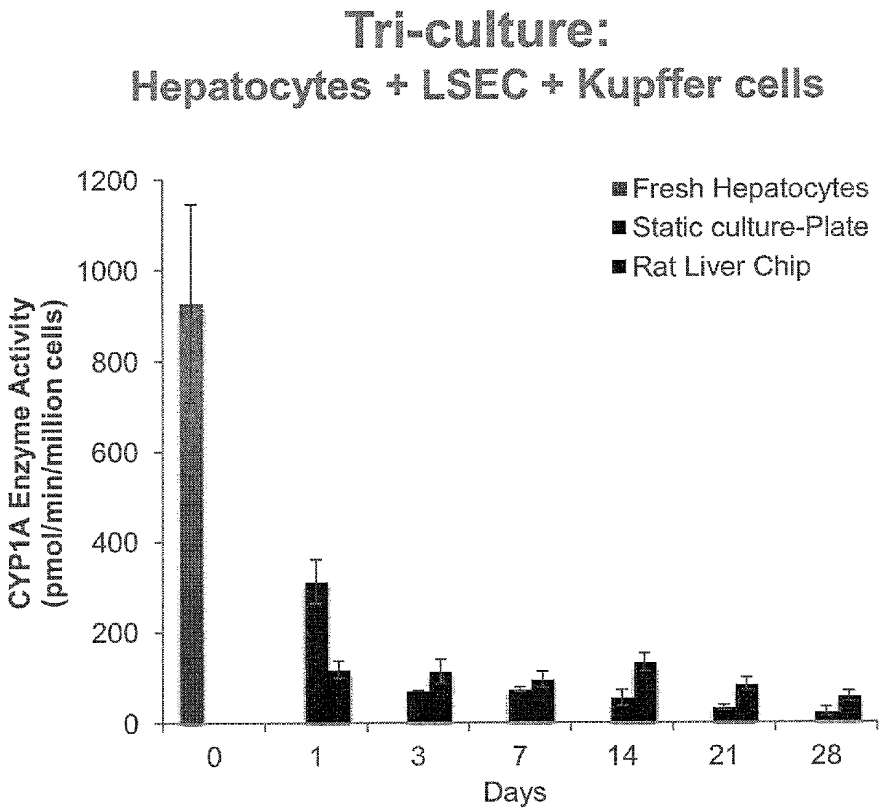
FIG. 31



emulate

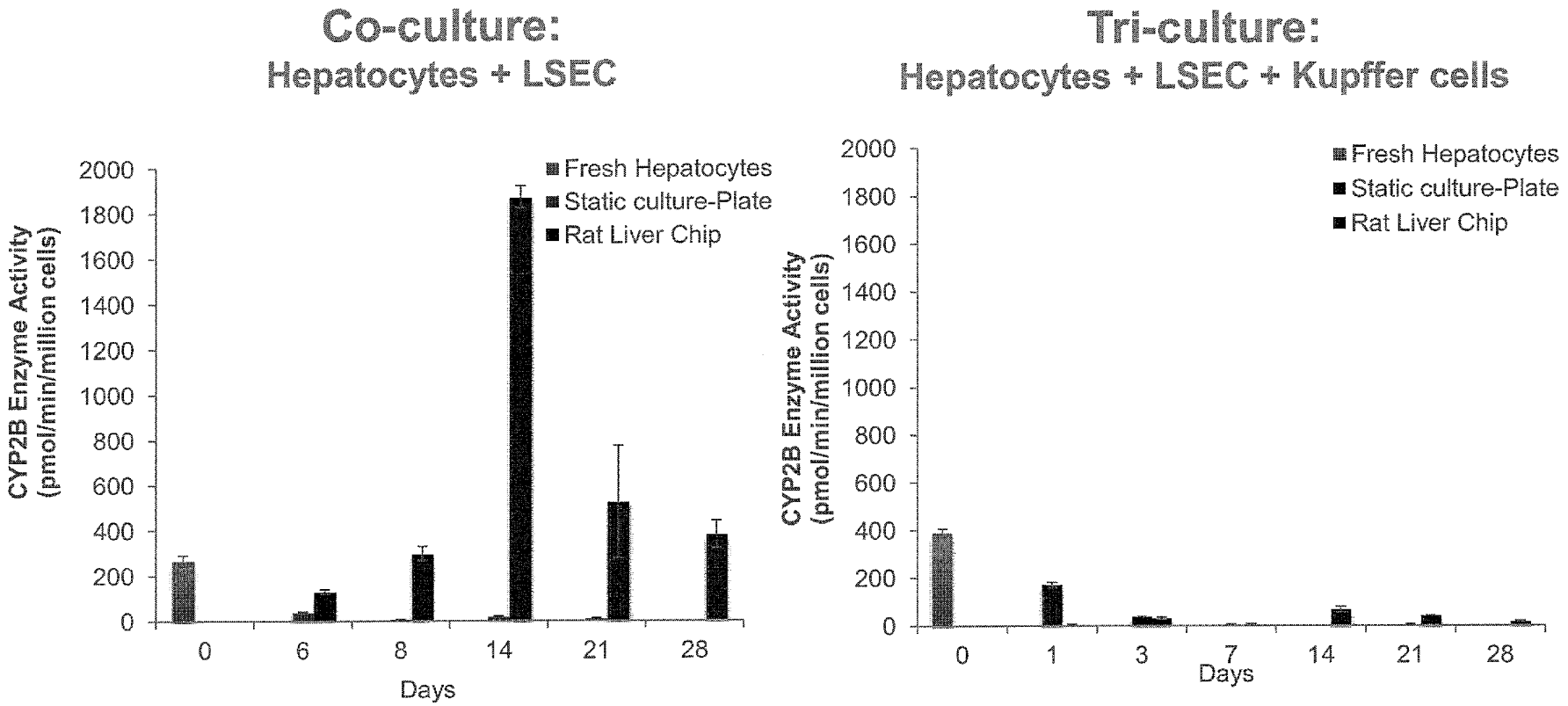
FIG. 32





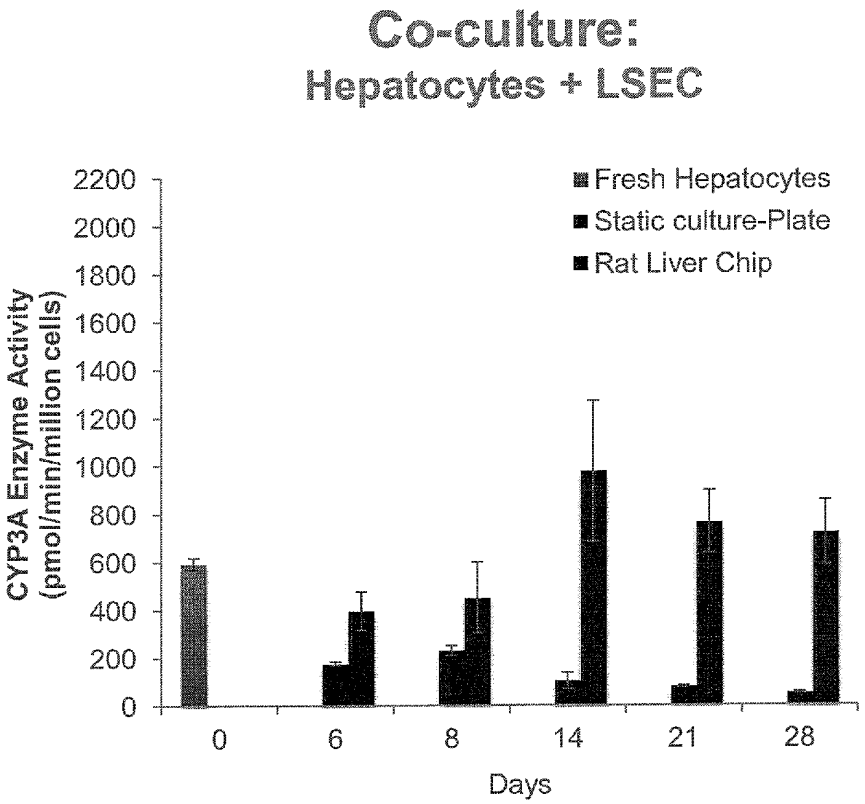
emulate

FIG. 33



emulate

FIG. 34


Co-culture:
Hepatocytes + LSEC
CYP3A Enzyme Activity (pmol/min/million cells)
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Fresh Hepatocytes
Static culture-Plate
Rat Liver Chip
0
6
8
14
21
28
Days

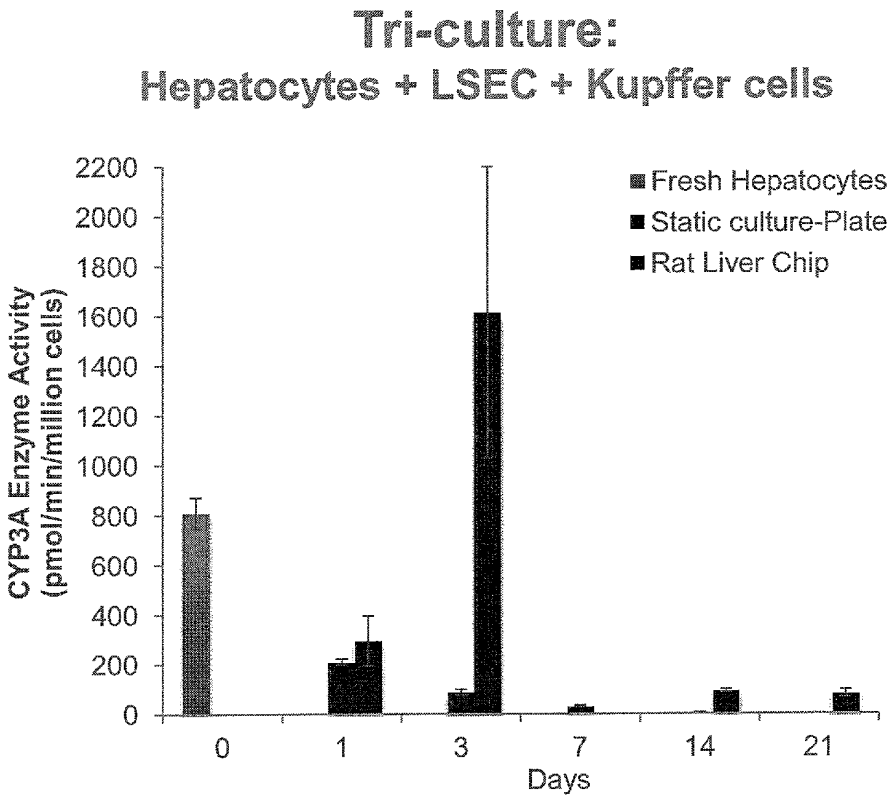
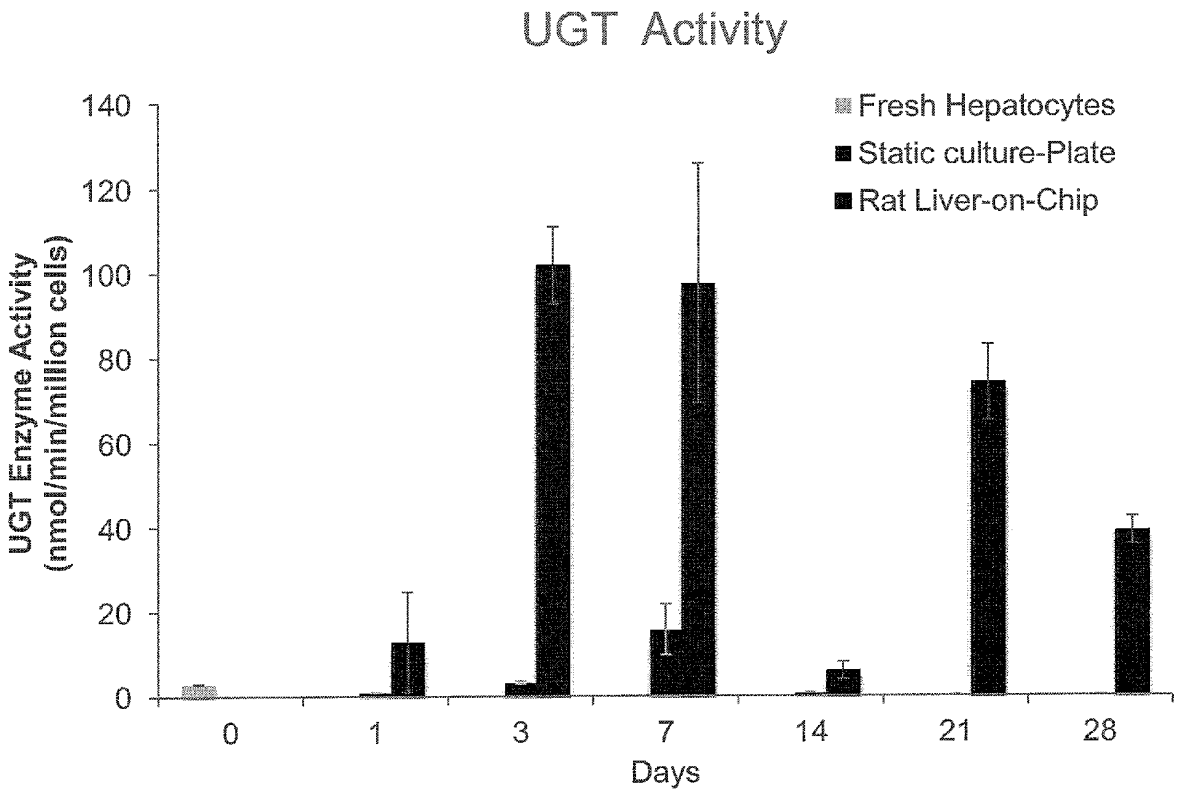

Tri-culture:
Hepatocytes + LSEC + Kupffer cells
CYP3A Enzyme Activity (pmol/min/million cells)
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Fresh Hepatocytes
Static culture-Plate
Rat Liver Chip
0
1
3
7
14
21
Days

FIG. 35



emulate

FIG. 36

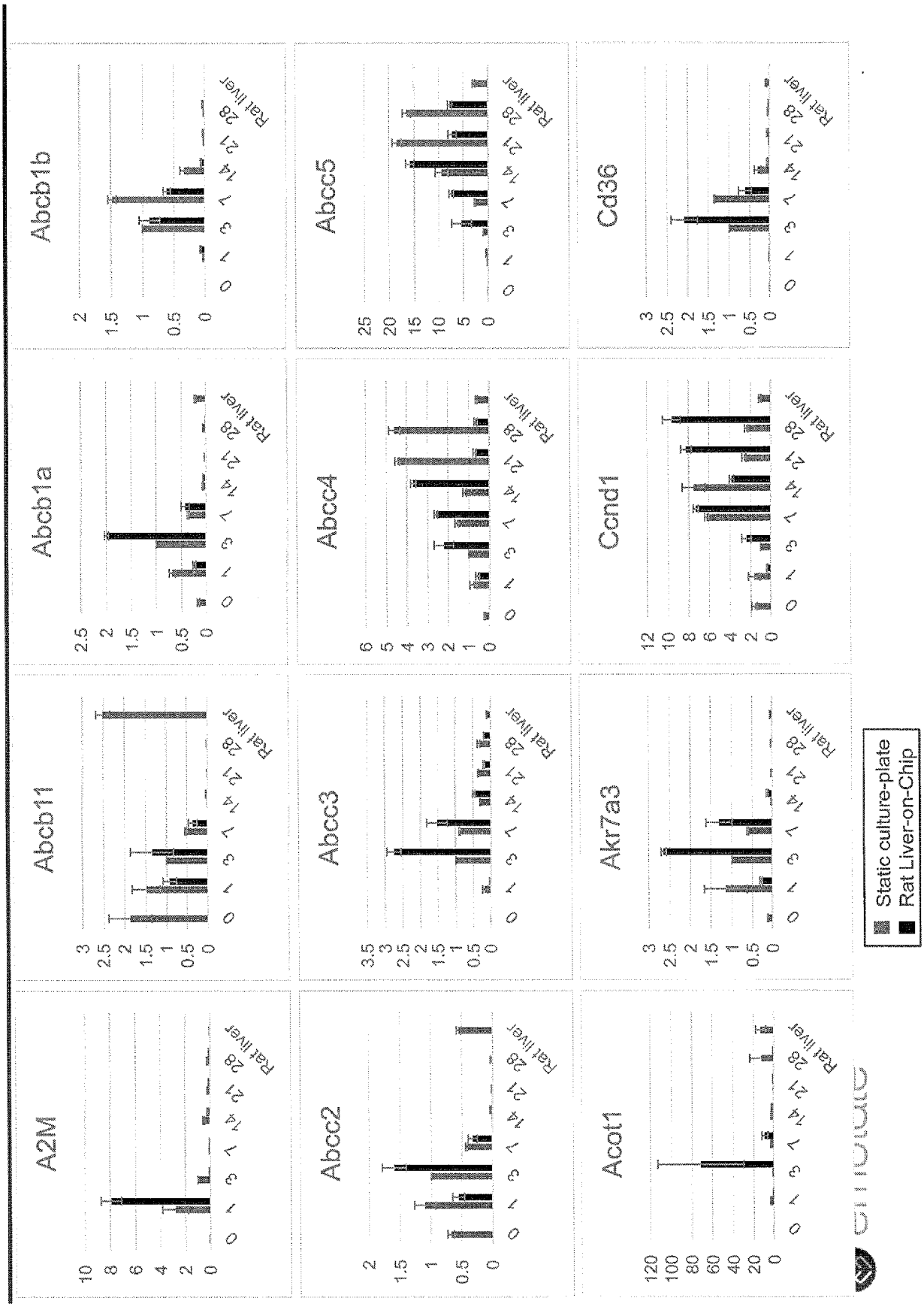

A2M
Abcb11
Abcb1a
Abcb1b
Abcc2
Abcc3
Abcc4
Abcc5
Acot1
Akr7a3
Ccnd1
Cd36
Rat liver
0 1 3 7 14 21 28
Static culture-plate
Rat Liver-on-Chip

FIG. 37

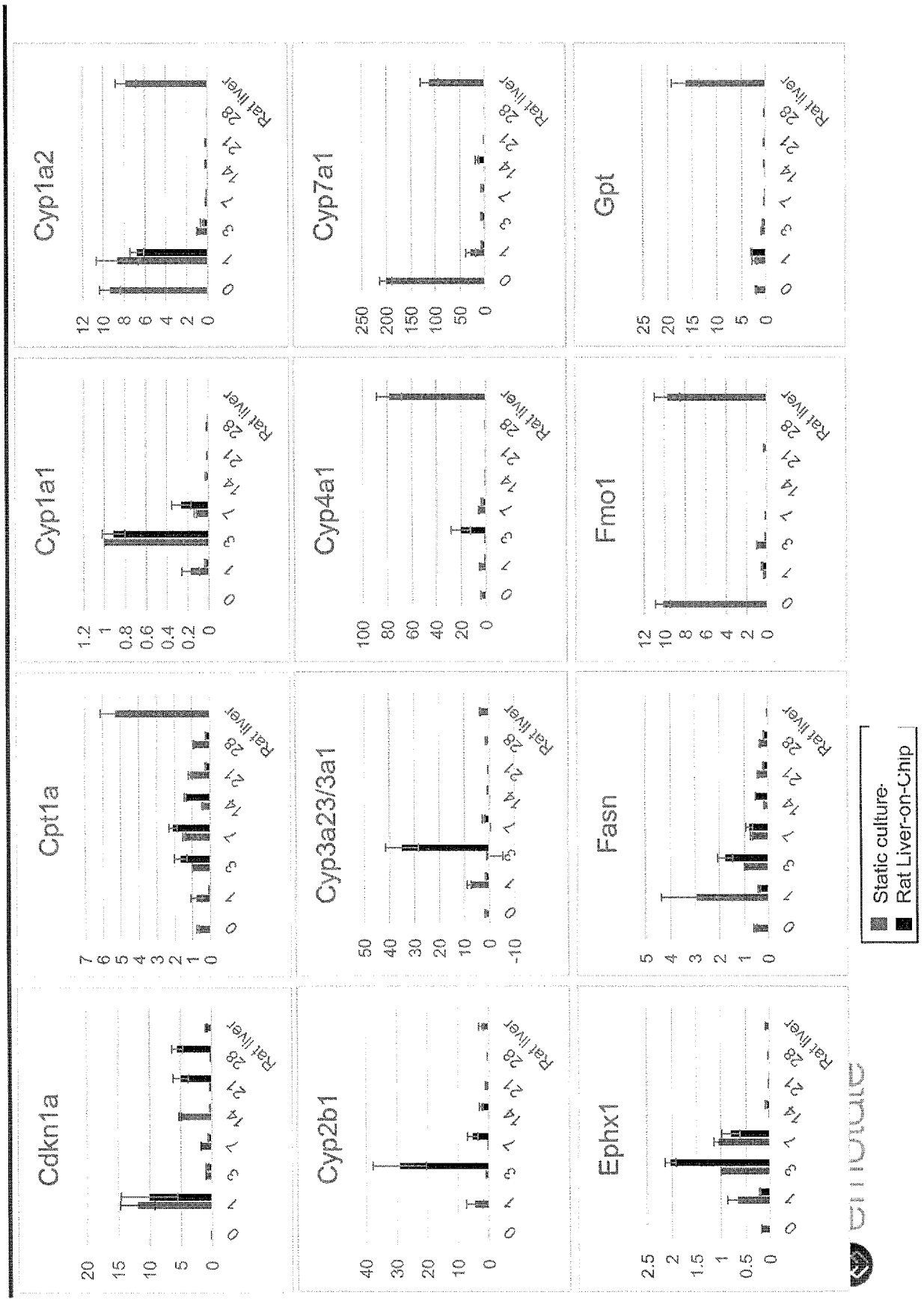

Cyp1a2
Cyp1a1
Cyp7a1
Gpt
Cpt1a
Cyp4a1
Fmo1
Cdkn1a
Cyp3a23/3a1
Fasn
Cyp2b1
Ephx1
Rat liver
0
1
3
7
14
21
28
Static culture
Rat Liver-on-Chip

FIG. 38

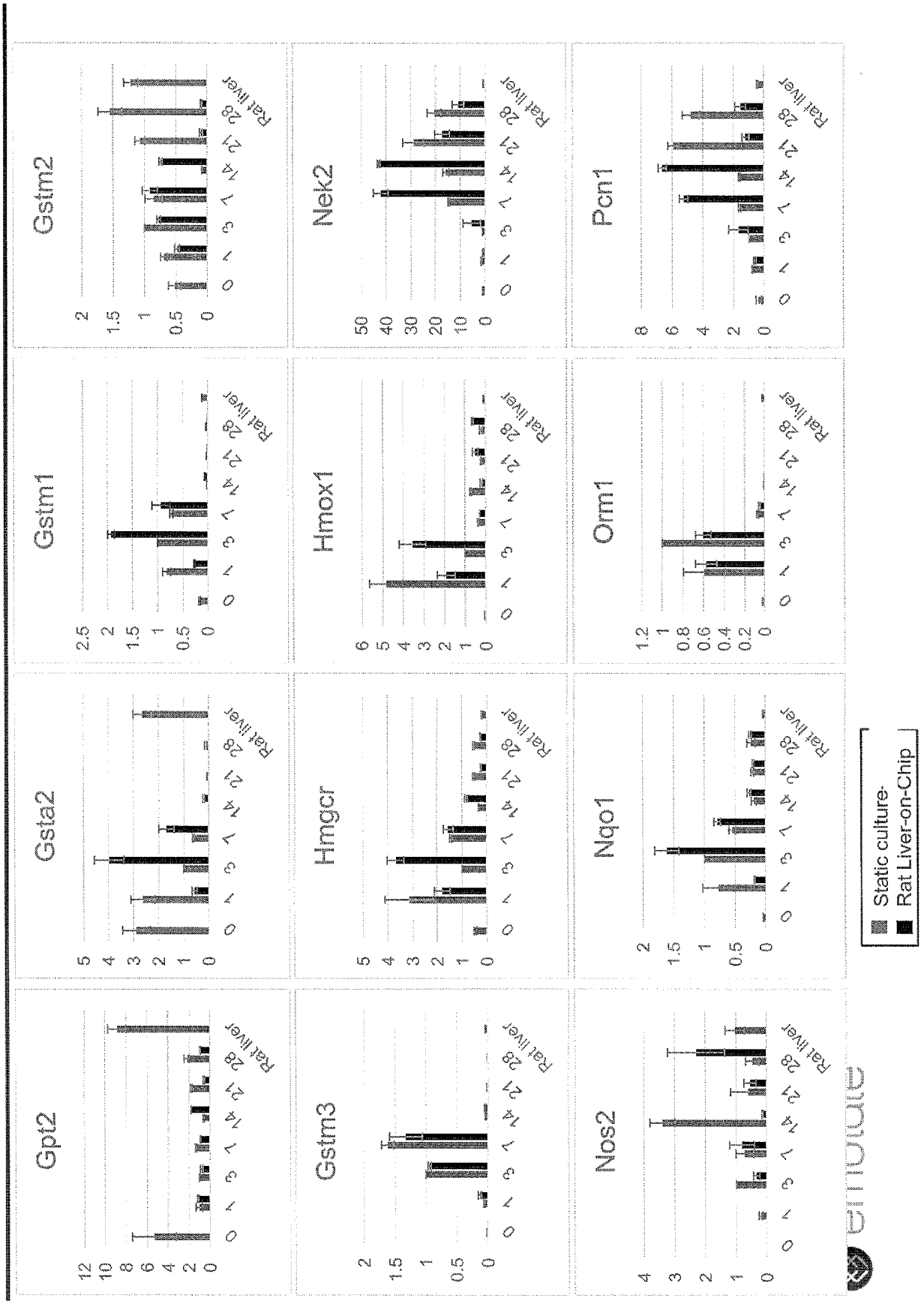

Gpt2
Gsta2
Gstm1
Gstm2
Gstm3
Hmgcr
Hmox1
Nek2
Nos2
Nqo1
Orm1
Pcn1
Rat liver
0
1
3
7
14
21
28
Static culture
Rat Liver-on-Chip

FIG. 39

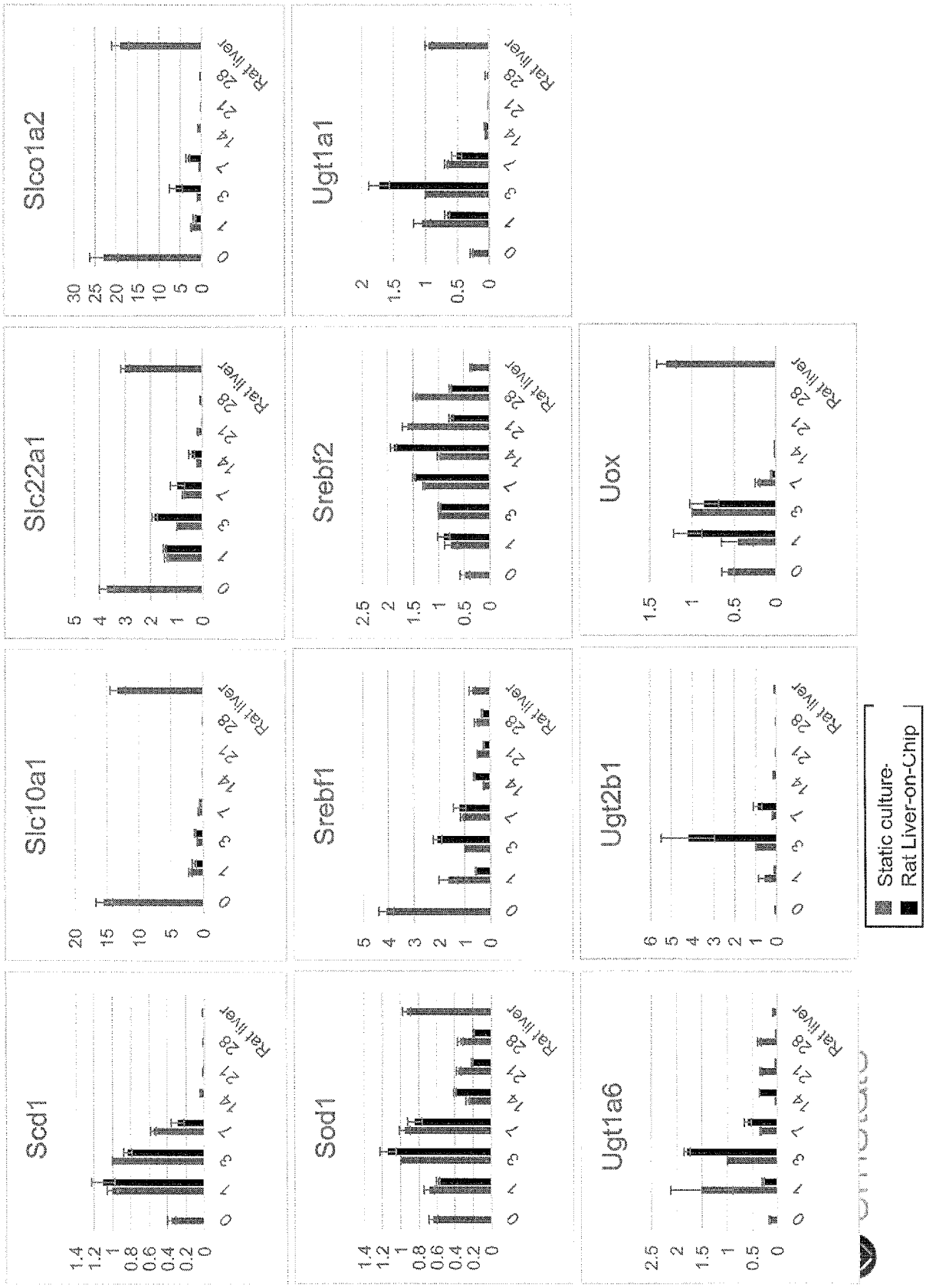
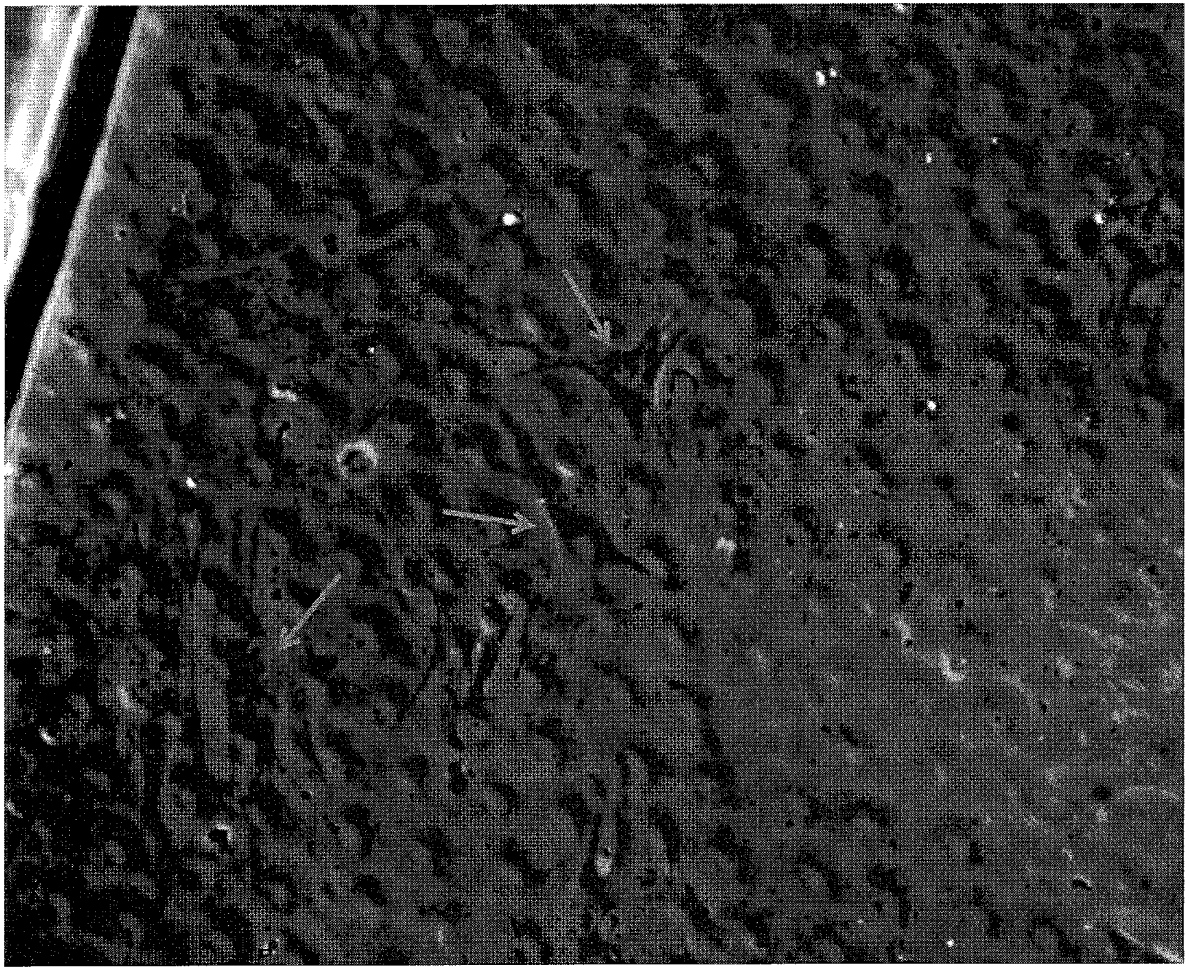

Scd1
Slc10a1
Slc22a1
Slco1a2
Sod1
Srebf1
Srebf2
Ugt1a1
Ugt1a6
Ugt2b1
Uox
Rat liver
0
1
3
7
14
21
28
Static culture
Rat Liver-on-Chip

FIG. 40



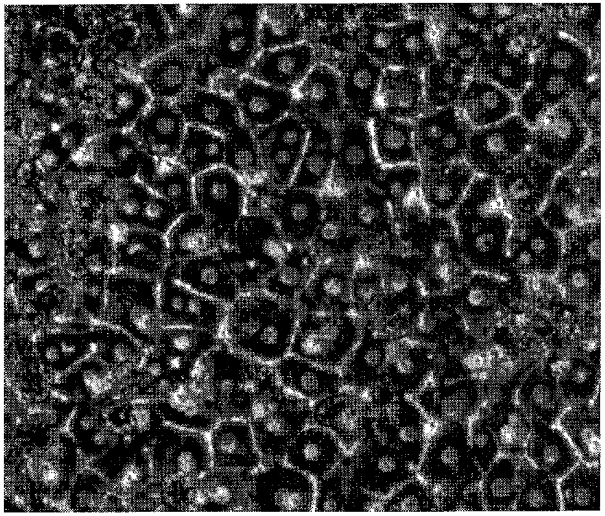
Stellate cells in the rat Liver-on-Chip at 3h post seeding

emulate

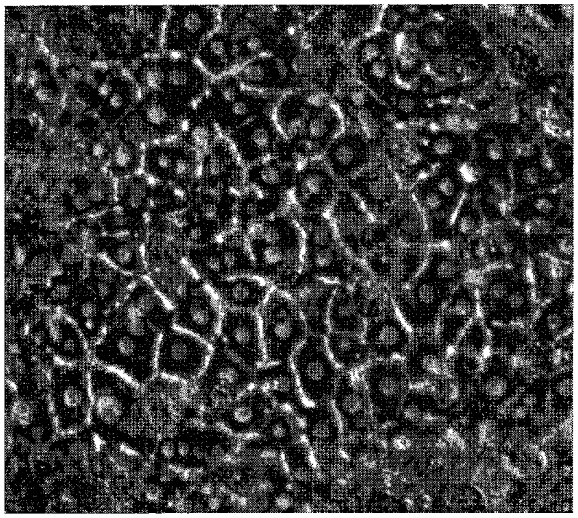
FIG. 41

Hepatocyte morphology in Day 4

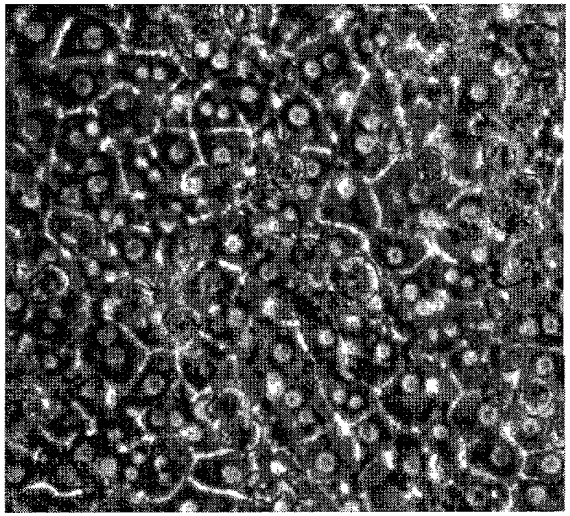
Co-culture



Tri-culture

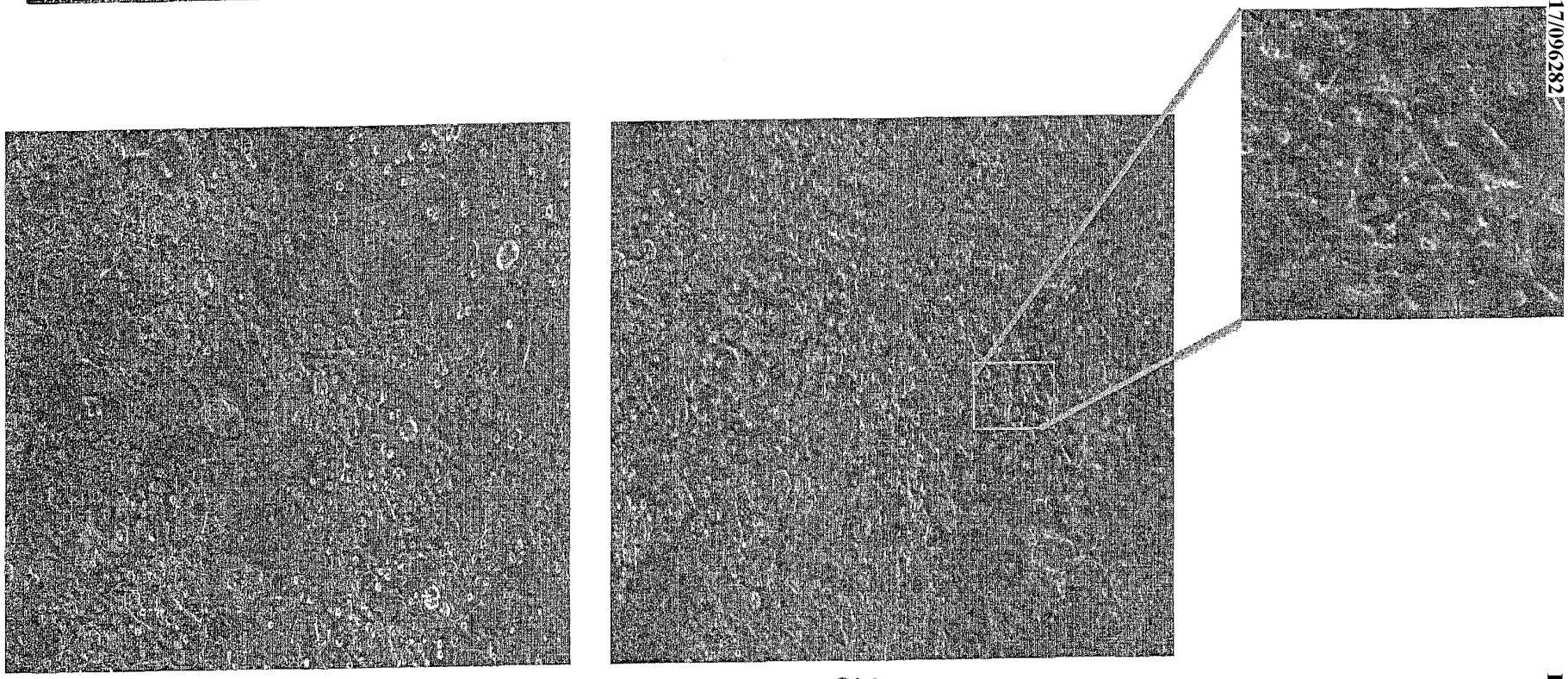


Quadruple-culture



emulate

FIG. 42



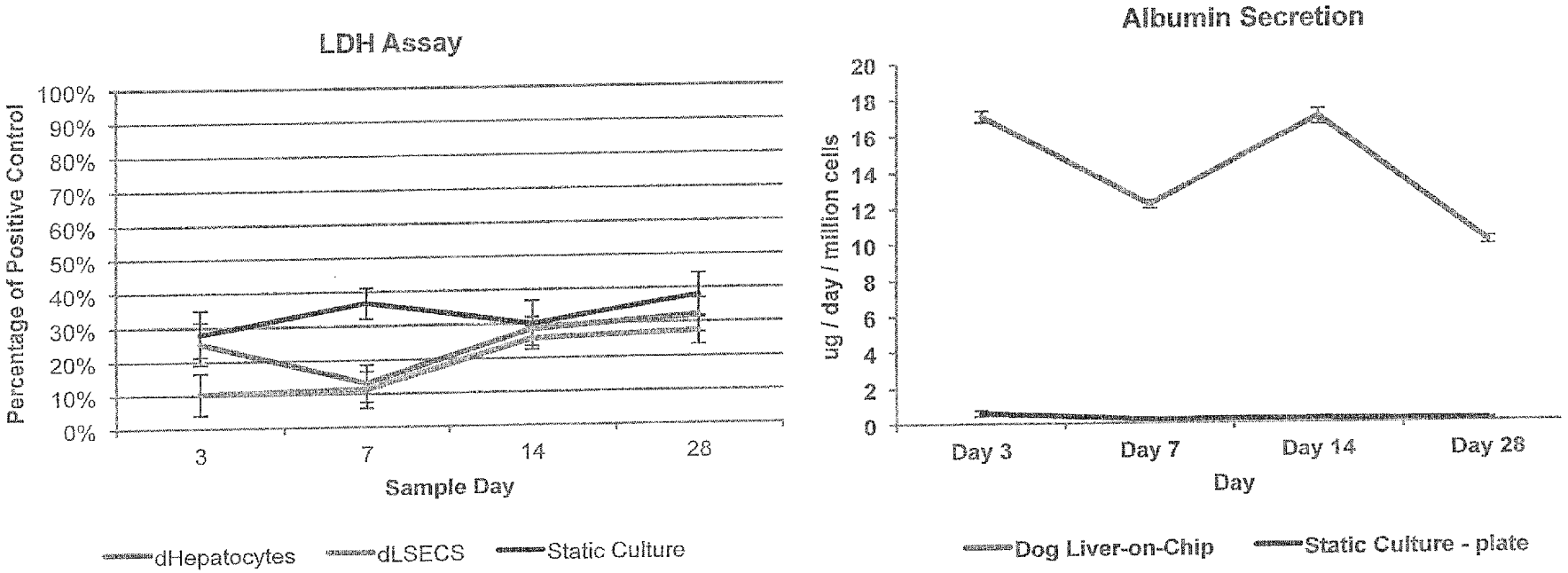
Static Culture dHepatocytes
Fresh – Day 14

Dog Liver-on-Chip
Fresh – Day 14

Dog Liver-on-chip shows correct hepatocyte morphology whereas, hepatocytes in static culture are beginning to flatten out

emulate

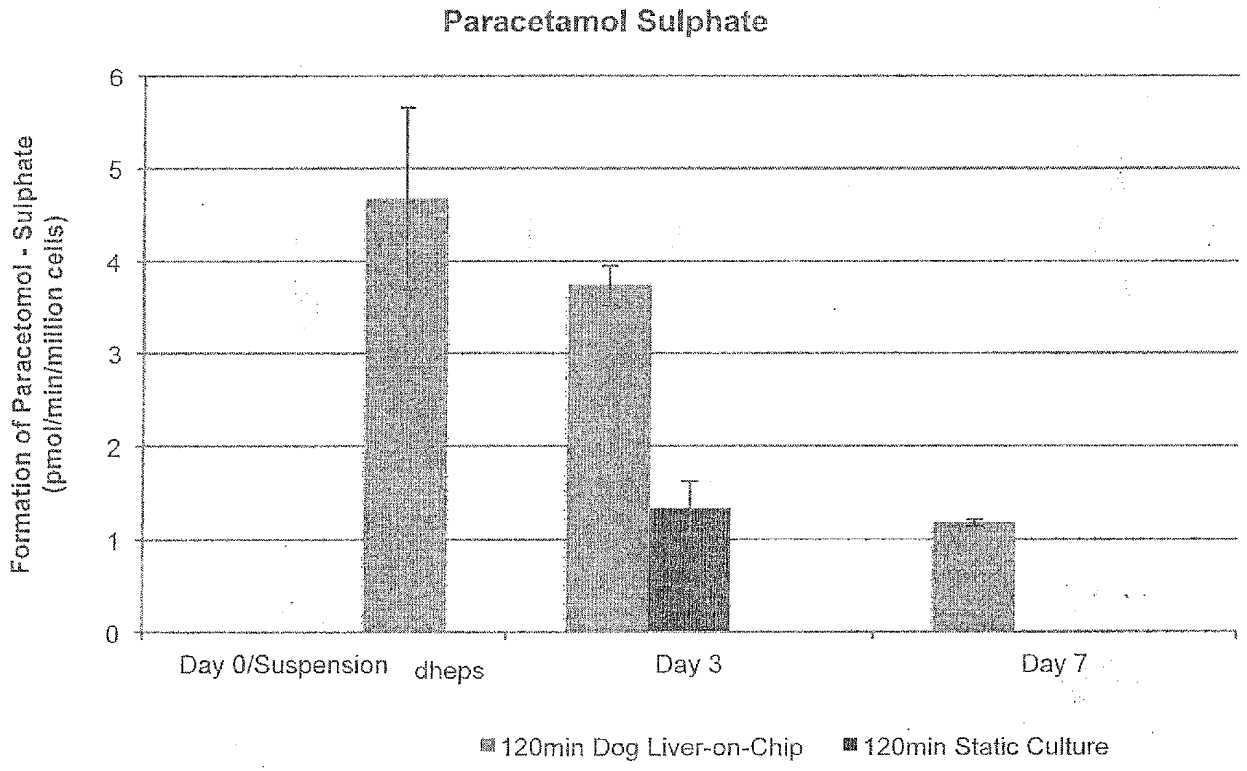
FIG. 43



Dog Liver-on-chip releases lower levels of LDH for 4 weeks compared to static culture
Dog Liver-on-Chip shows improved albumin secretion compared to static culture

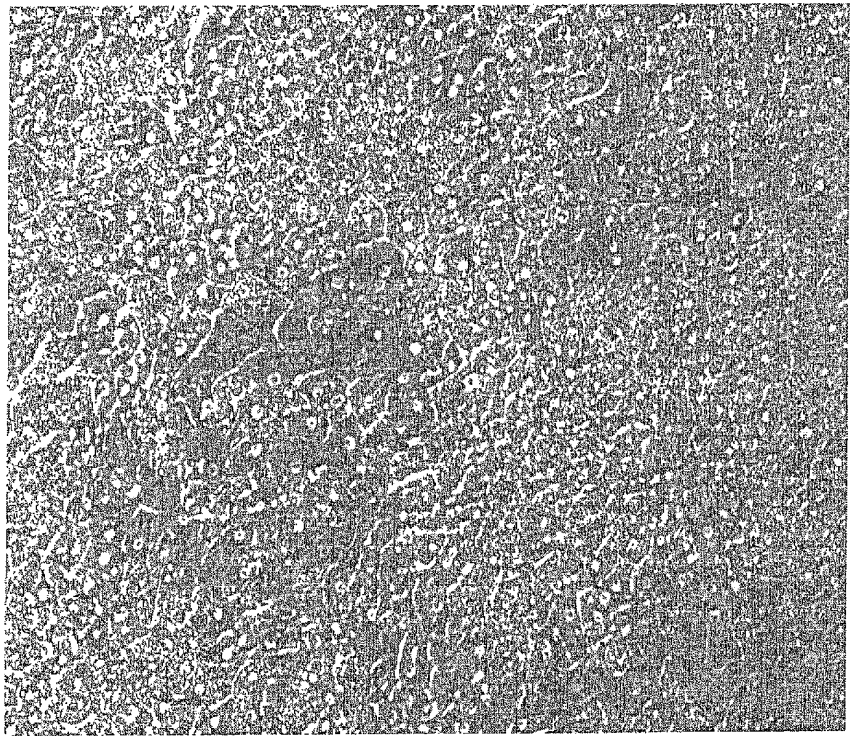
emulate

FIG.44

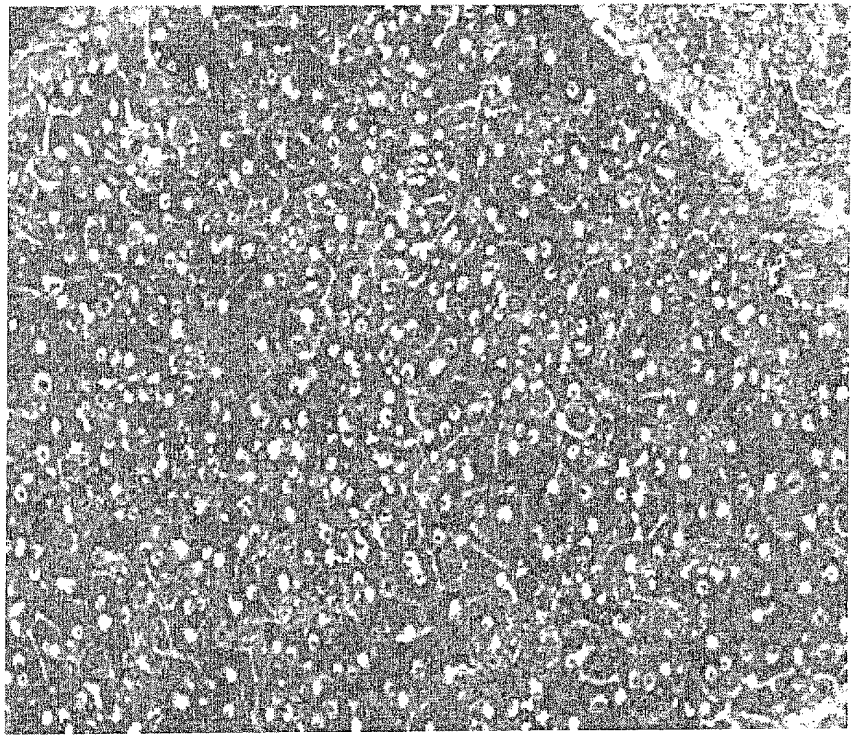


Dog Liver-on-Chip shows higher levels of phase II enzyme activity for 1 week when compared to conventional static cultured hepatocytes

FIG.45



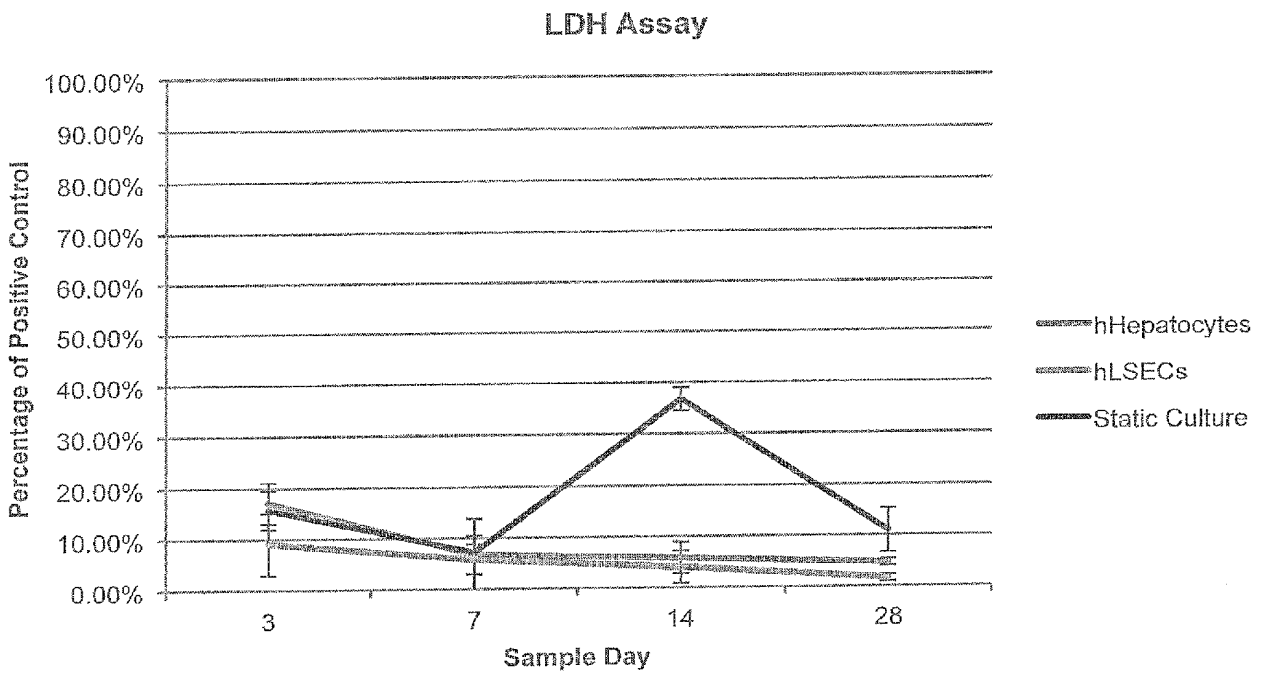
Static Culture hHepatocytes
Day 14



Human Liver-on-Chip
Day 14

Human Liver-on-Chip shows healthy morphology after 2 weeks whereas hepatocytes in static culture begi[redacted]ght junctions and secrete cellular waste

FIG. 46



Human Liver-on-Chip maintains low LDH levels for 28 days whereas hepatocytes in static culture show an increase in LDH at day 14 indicating poor cell health

emulate

FIG. 47

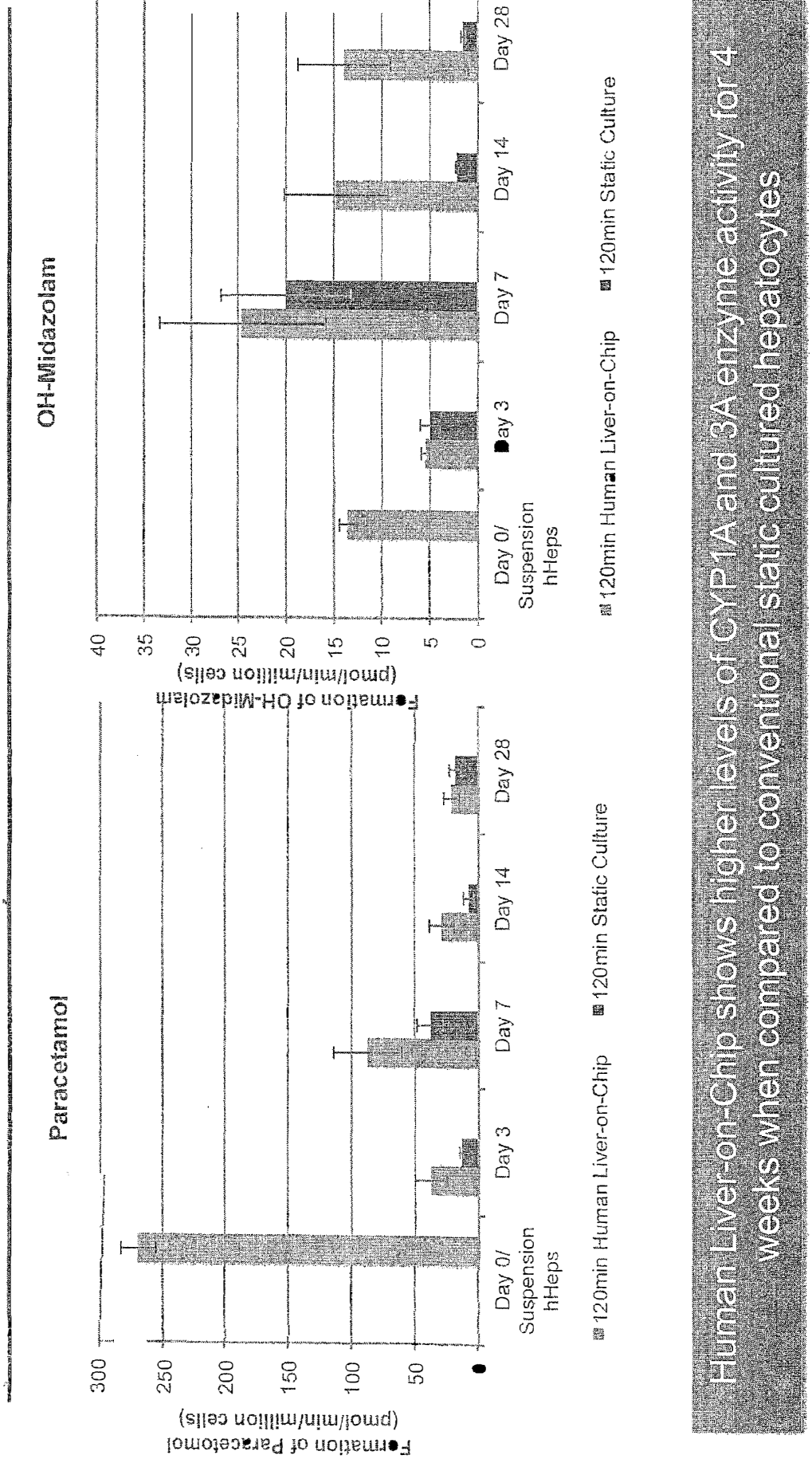
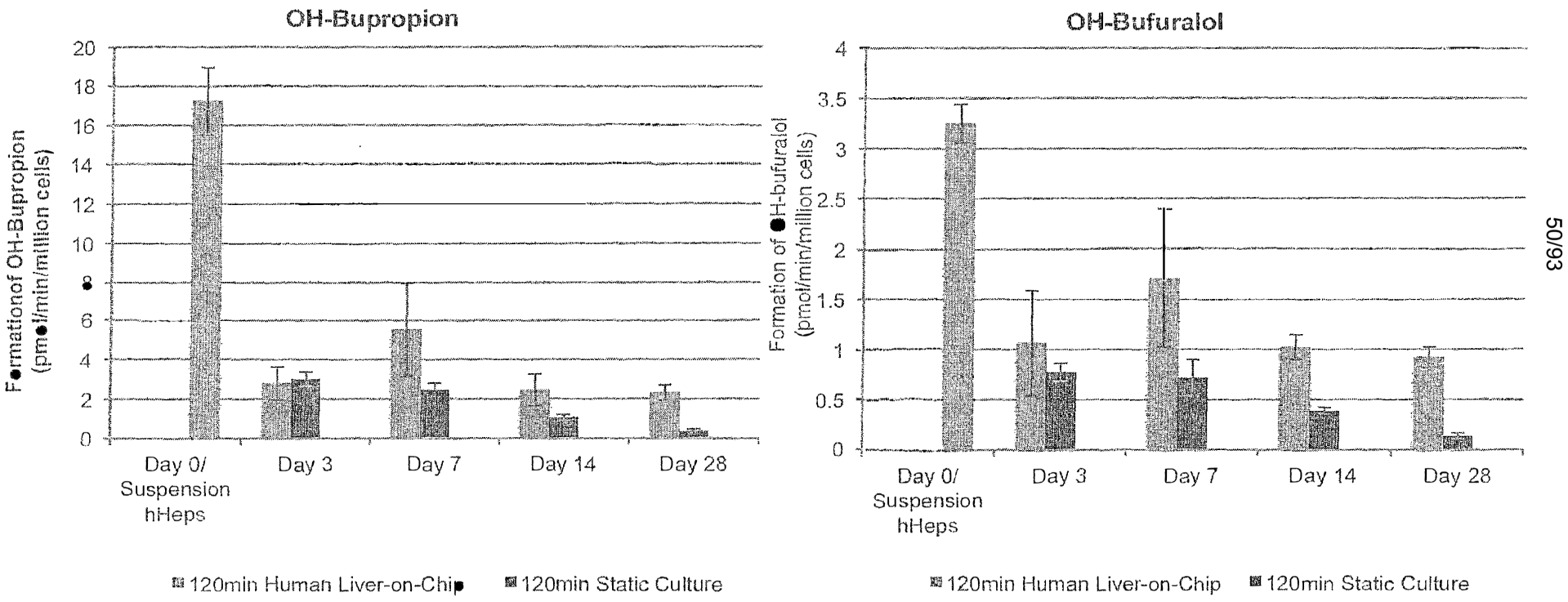
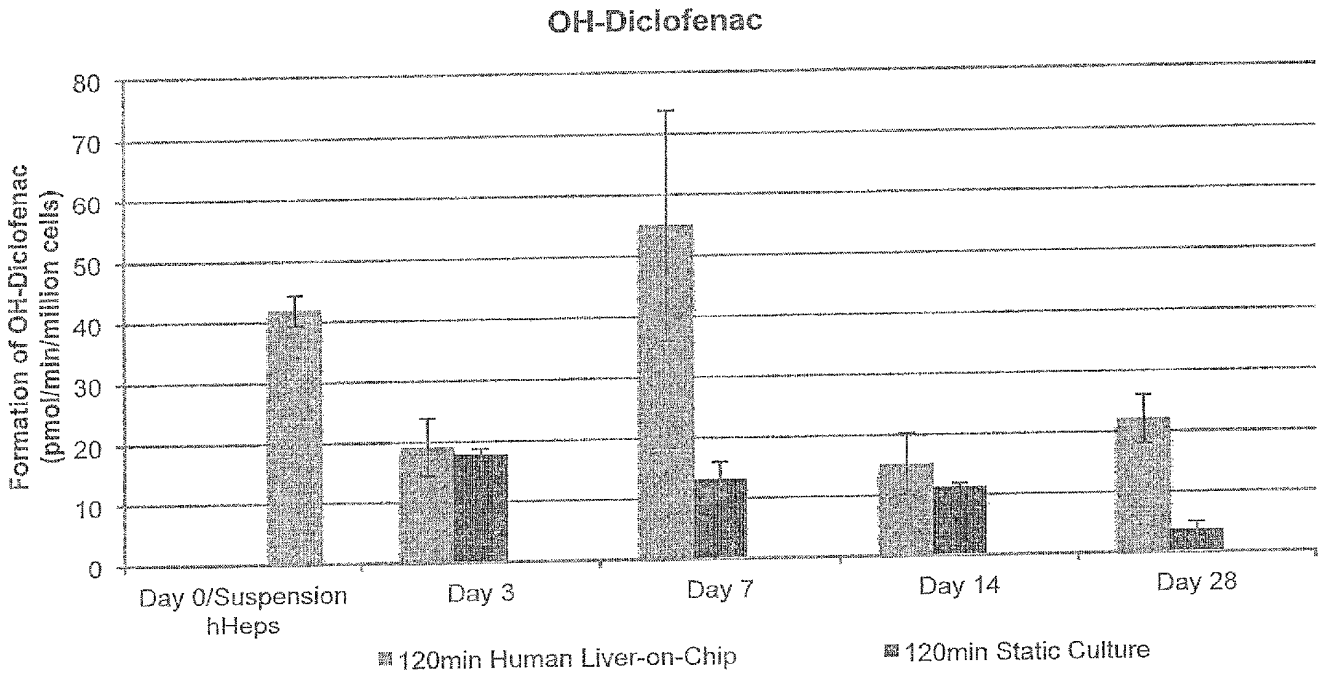

Paracetamol
Formation of Paracetomol (pmol/min/million cells)
300
250
200
150
100
50
0
Day 0/ Suspension hHeps
Day 3
Day 7
Day 14
Day 28
OH-Midazolam
Formation of OH-Midazolam (pmol/min/million cells)
40
35
30
25
20
15
10
5
0
120min Human Liver-on-Chip
120min Static Culture
Human Liver-on-Chip shows higher levels of CYP1A and 3A enzyme activity for 4 weeks when compared to conventional static cultured hepatocytes

FIG. 48



Human Liver-on-Chip shows higher levels of CYP2B and 2D enzyme activity for 4 weeks when compared to conventional static cultured hepatocytes

FIG. 49



Human Liver-on-Chip shows improved levels of CYP2C enzyme activity for 4 weeks when compared to conventional static cultured hepatocytes

emulate

Figure 50

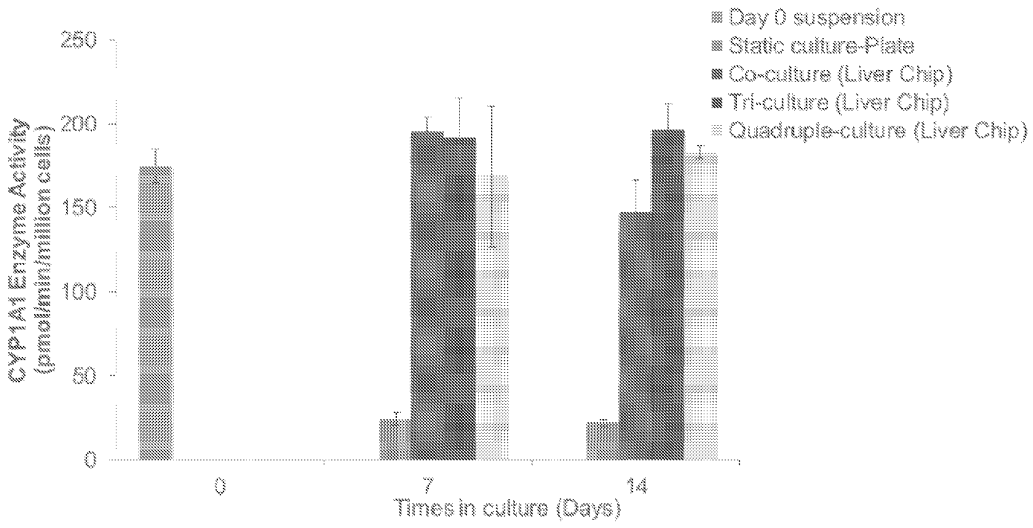

Day 0 suspension
Static culture-Plate
Co-culture (Liver Chip)
Tri-culture (Liver Chip)
Quadruple-culture (Liver Chip)
CYP1A1 Enzyme Activity
(pmol/min/million cells)
250
200
150
100
50
0
0
7
14
Times in culture (Days)

Figure 51

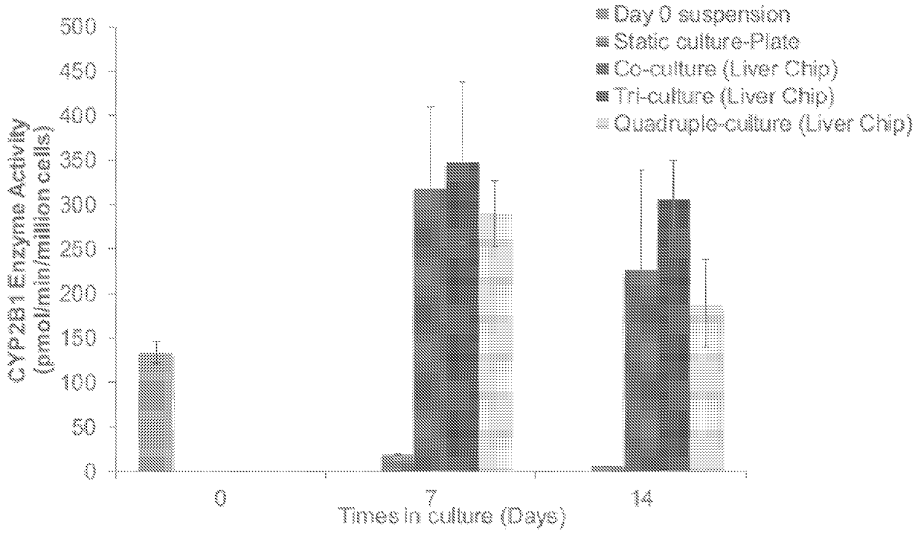

Day 0 suspension
Static culture-Plate
Co-culture (Liver Chip)
Tri-culture (Liver Chip)
Quadruple-culture (Liver Chip)
CYP2B1 Enzyme Activity (pmol/min/million cells)
500
450
400
350
300
250
200
150
100
50
0
0
7
14
Times in culture (Days)

Figure 52

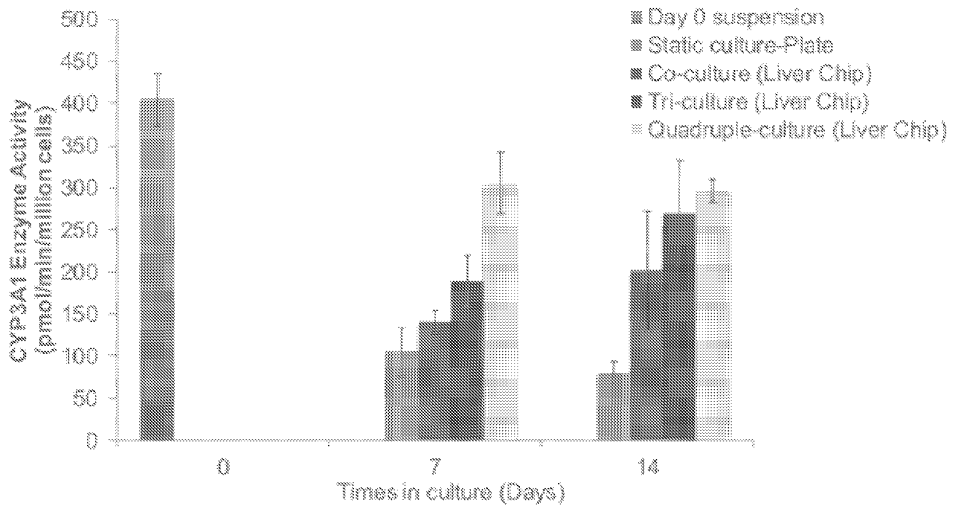

CYP3A1 Enzyme Activity
(pmol/min/million cells)
500
450
400
350
300
250
200
150
100
50
0
0
7
14
Times in culture (Days)
Day 0 suspension
Static culture-Plate
Co-culture (Liver Chip)
Tri-culture (Liver Chip)
Quadruple-culture (Liver Chip)

Figure 53

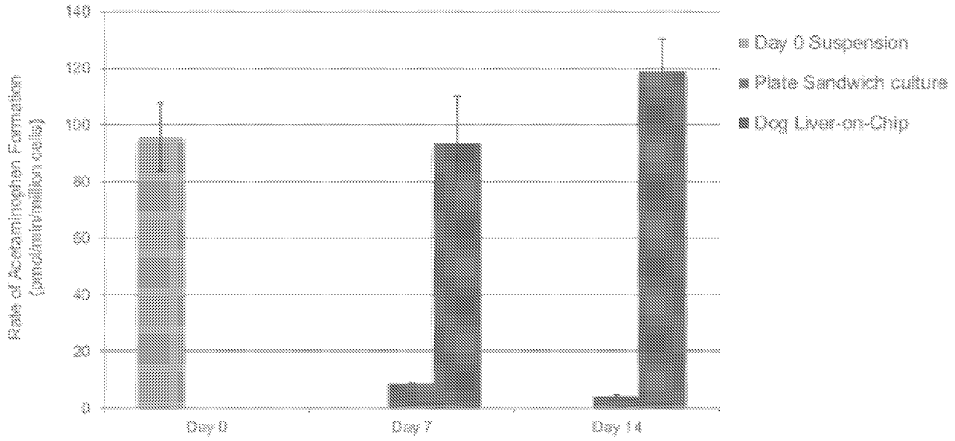

Rate of Acetaminophen Formation (pmol/min/million cells)
140
120
100
80
60
40
20
0
Day 0
Day 7
Day 14
Day 0 Suspension
Plate Sandwich culture
Dog Liver-on-Chip

Figure 54

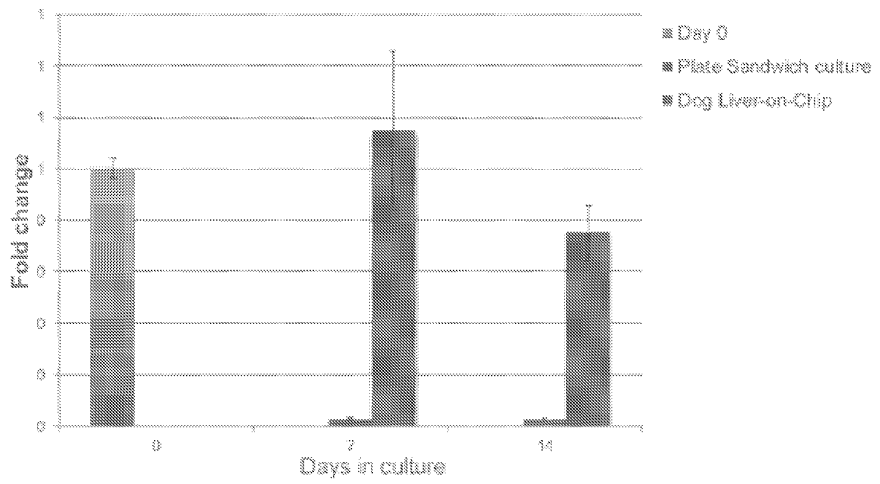
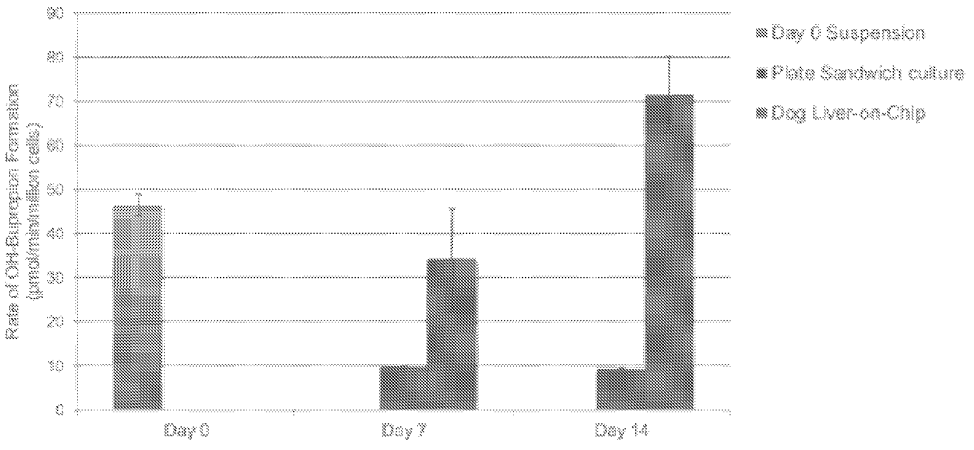

Fold change
Days in culture
0
7
14
Day 0
Plate Sandwich culture
Dog Liver-on-Chip

Figure 55


Rate of OH Bupropion Formation (pmol/min/million cells)
0
10
20
30
40
50
60
70
80
90
Day 0
Day 7
Day 14
Day 0 Suspension
Plate Sandwich culture
Dog Liver-on-Chip

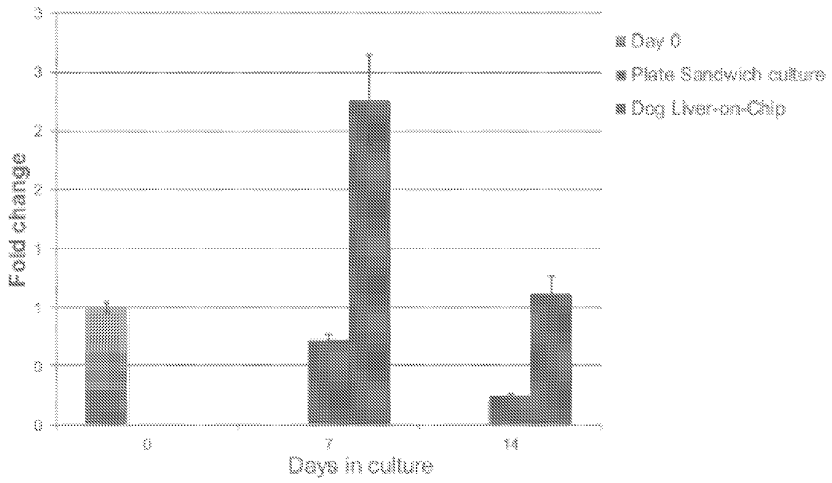

Fold change
Days in culture
0
7
14
Day 0
Plate Sandwich culture
Dog Liver-on-Chip

Figure 56

Figure 57

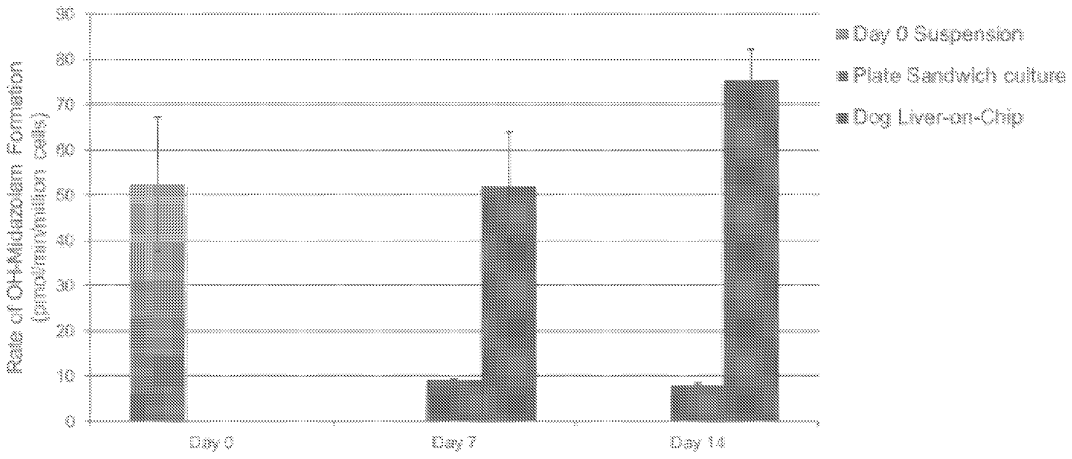

Rate of OH-Midazolam Formation
(pmol/min/million cells)
0
10
20
30
40
50
60
70
80
90
Day 0
Day 7
Day 14
Day 0 Suspension
Plate Sandwich culture
Dog Liver-on-Chip

Figure 58

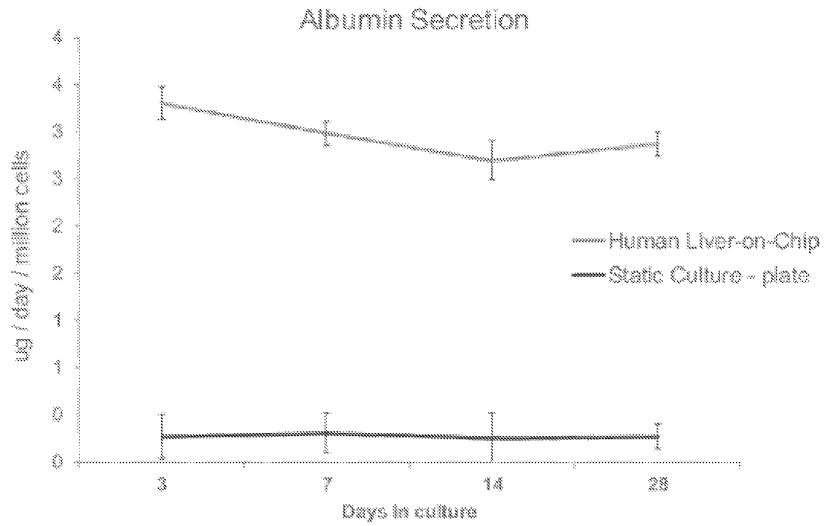

Albumin Secretion
ug / day / million cells
0
1
2
3
4
3
7
14
28
Days in culture
Human Liver-on-Chip
Static Culture - plate

Figure 59

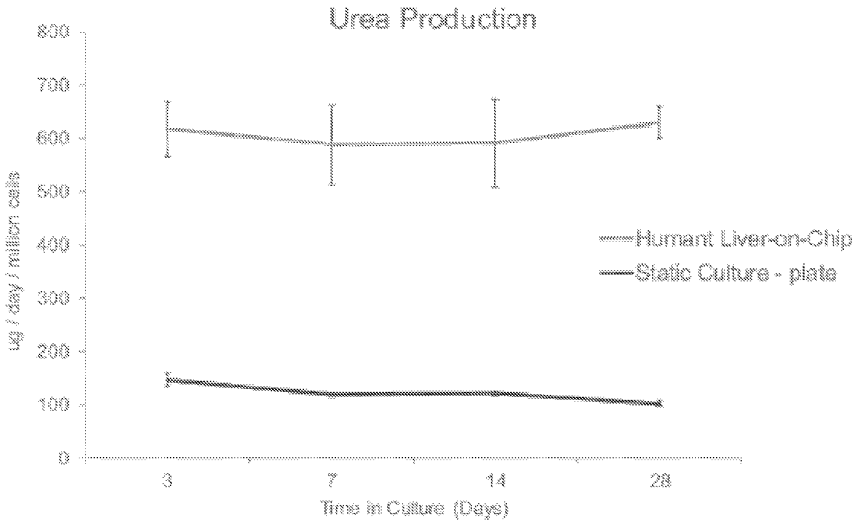
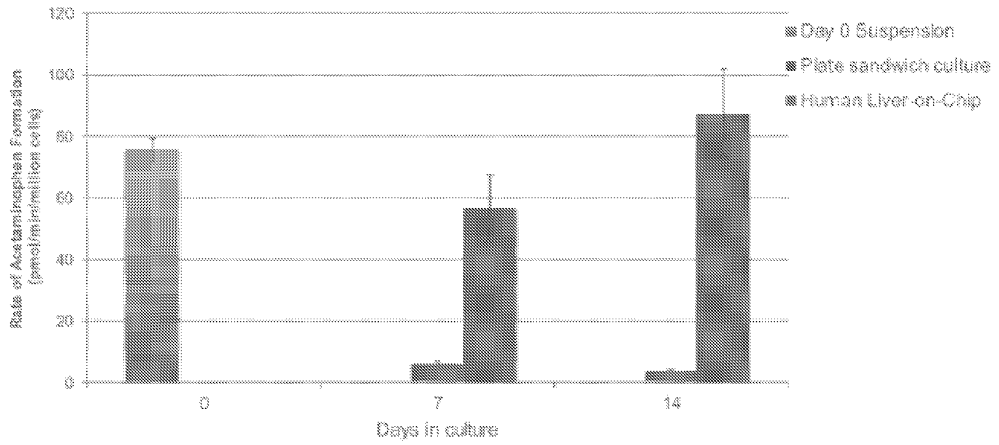

Urea Production
800
700
600
500
400
300
200
100
0
ug / day / million cells
3
7
14
28
Time in Culture (Days)
Humant Liver-on-Chip
Static Culture - plate

Figure 60


120
100
80
60
40
20
0
Rate of Acetaminophen Formation (pmol/min/million cells)
0
7
14
Days in culture
Day 0 Suspension
Plate sandwich culture
Human Liver-on-Chip

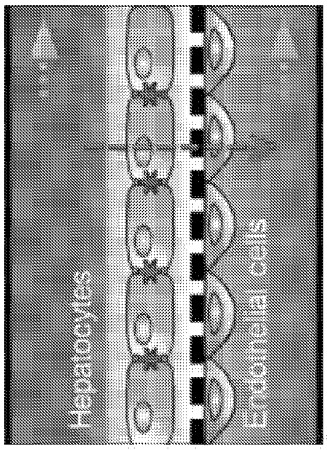


Figure 61

Figure 62

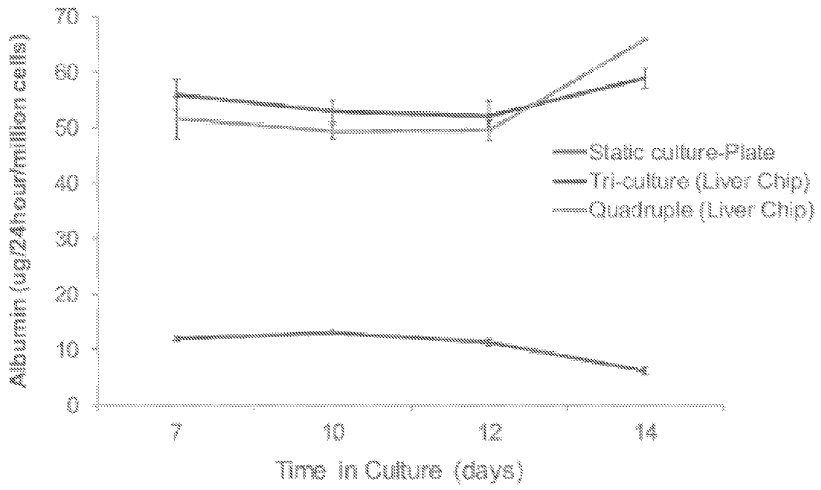

Albumin (ug/24hour/million cells)
70
60
50
40
30
20
10
0
7
10
12
14
Time in Culture (days)
Static culture-Plate
Tri-culture (Liver Chip)
Quadruple (Liver Chip)

Figure 63

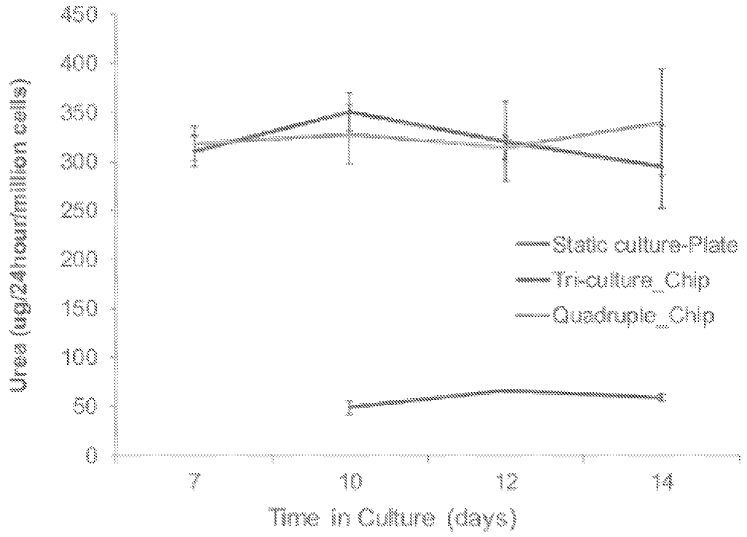

450
400
350
300
250
200
150
100
50
0
Urea (ug/24hour/million cells)
7
10
12
14
Time in Culture (days)
Static culture-Plate
Tri-culture_Chip
Quadruple_Chip

Figure 64

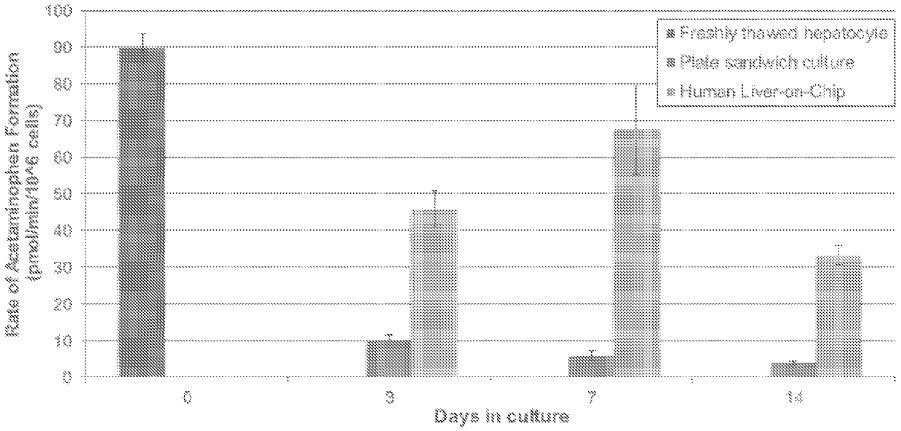

Rate of Acetaminophen Formation (pmol/min/10^6 cells)
100
90
80
70
60
50
40
30
20
10
0
0
3
7
14
Days in culture
Freshly thawed hepatocyte
Plate sandwich culture
Human Liver-on-Chip

Figure 65

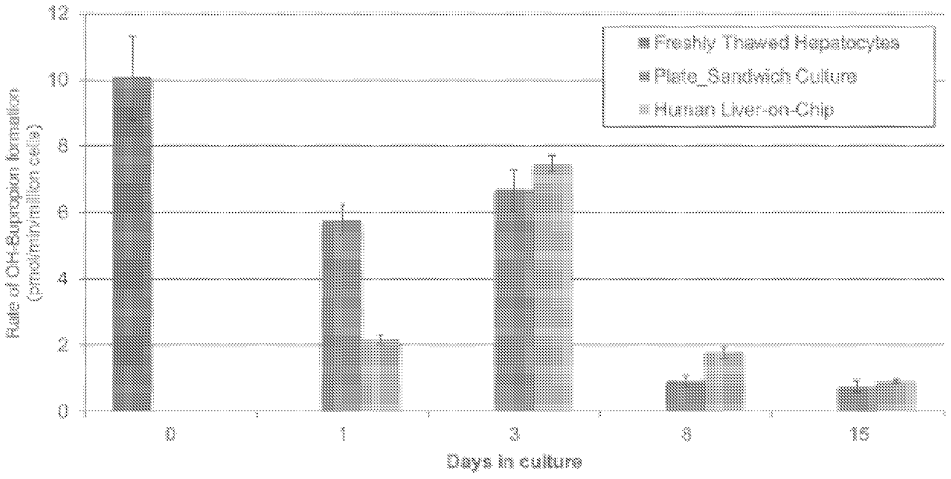

Freshly Thawed Hepatocytes
Plate_Sandwich Culture
Human Liver-on-Chip
Rate of OH-Bupropion formation (pmol/min/million cells)
0
2
4
6
8
10
12
0
1
3
8
15
Days in culture

Figure 66

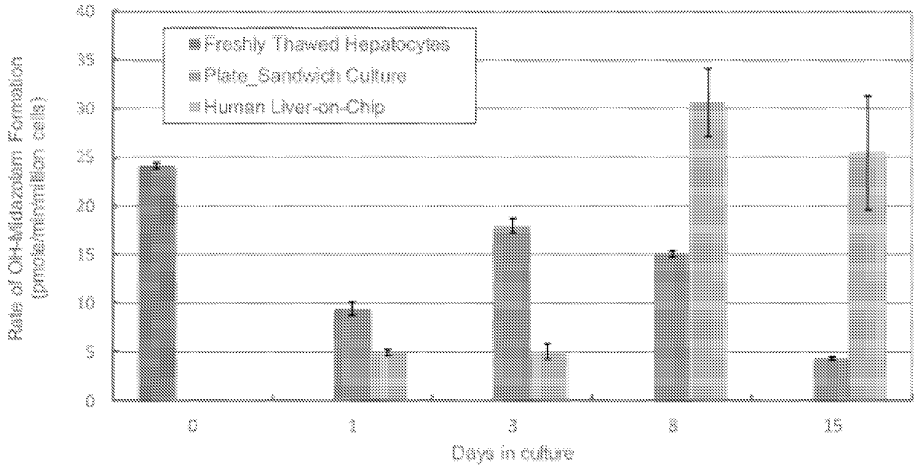
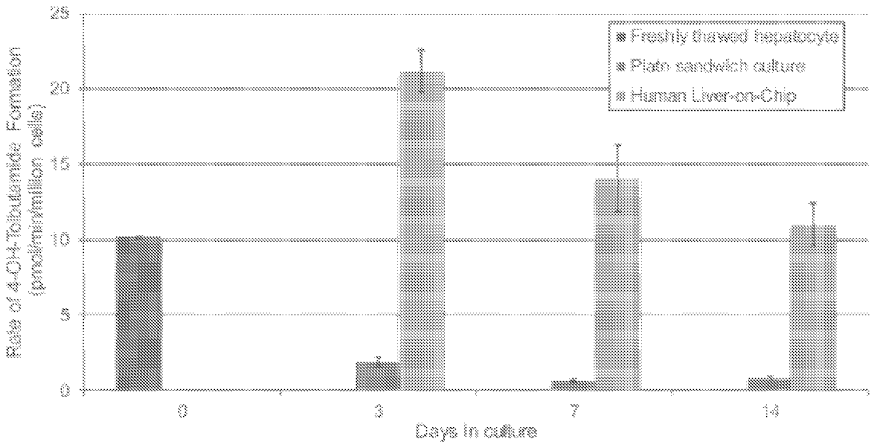

Rate of OH-Midazolam Formation (pmole/min/million cells)
Freshly Thawed Hepatocytes
Plate_Sandwich Culture
Human Liver-on-Chip
0
5
10
15
20
25
30
35
40
0
1
3
8
15
Days in culture

Figure 67


Rate of 4-OH-Tolbutamide Formation (pmol/min/million cells)
25
20
15
10
5
0
0
3
7
14
Days in culture
Freshly thawed hepatocyte
Plate sandwich culture
Human Liver-on-Chip

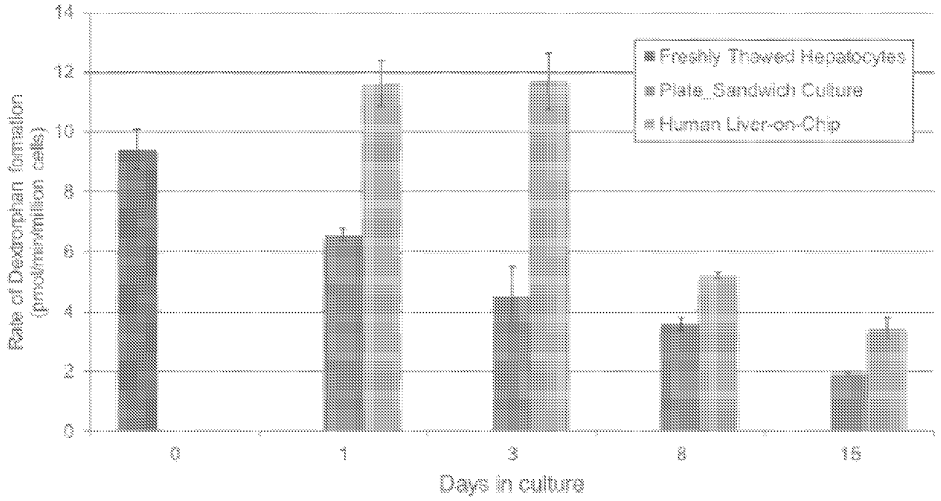

Freshly Thawed Hepatocytes
Plate_Sandwich Culture
Human Liver-on-Chip
Rate of Dexamphan formation (pmol/min/million cells)
0
2
4
6
8
10
12
14
0
1
3
8
15
Days in culture

Figure 68

Figure 69

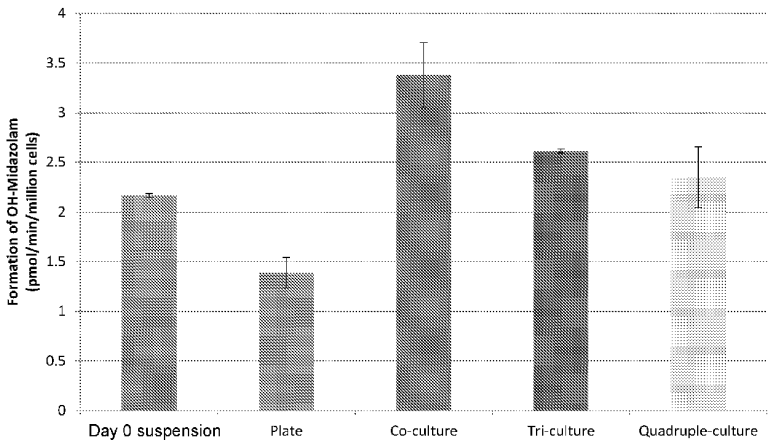
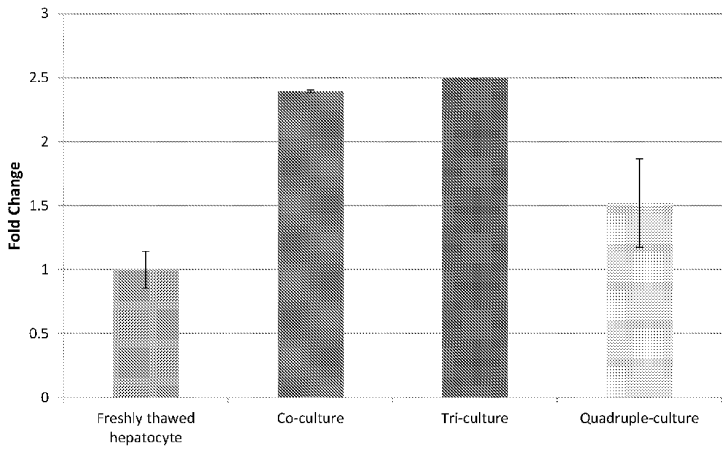

Formation of OH-Midazolam (pmol/min/million cells)
4
3.5
3
2.5
2
1.5
1
0.5
0
Day 0 suspension
Plate
Co-culture
Tri-culture
Quadruple-culture

Figure 70


3
2.5
2
1.5
1
0.5
0
Fold Change
Freshly thawed hepatocyte
Co-culture
Tri-culture
Quadruple-culture

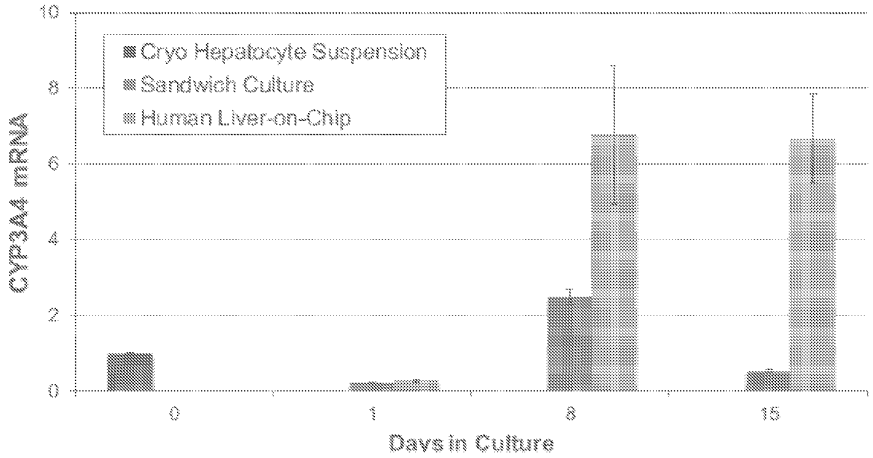

10
8
6
4
2
0
CYP3A4 mRNA
Cryo Hepatocyte Suspension
Sandwich Culture
Human Liver-on-Chip
0
1
8
15
Days in Culture

Figure 71

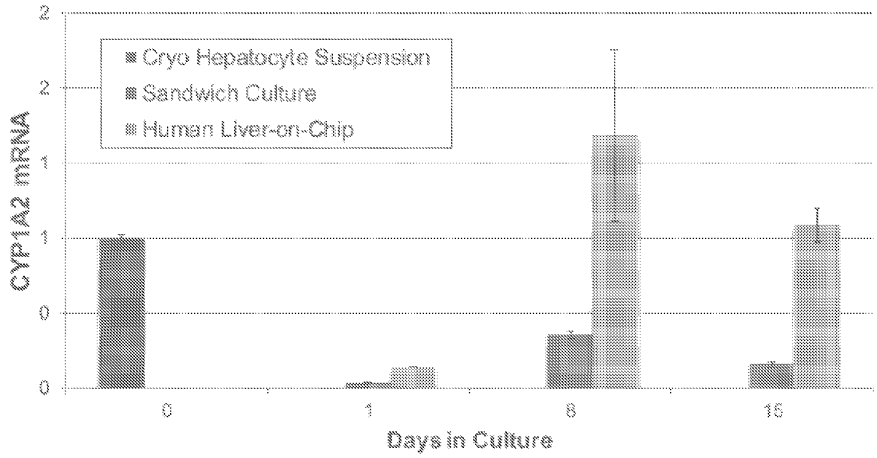

Cryo Hepatocyte Suspension
Sandwich Culture
Human Liver-on-Chip
CYP1A2 mRNA
Days in Culture
0
1
8
16

Figure 72

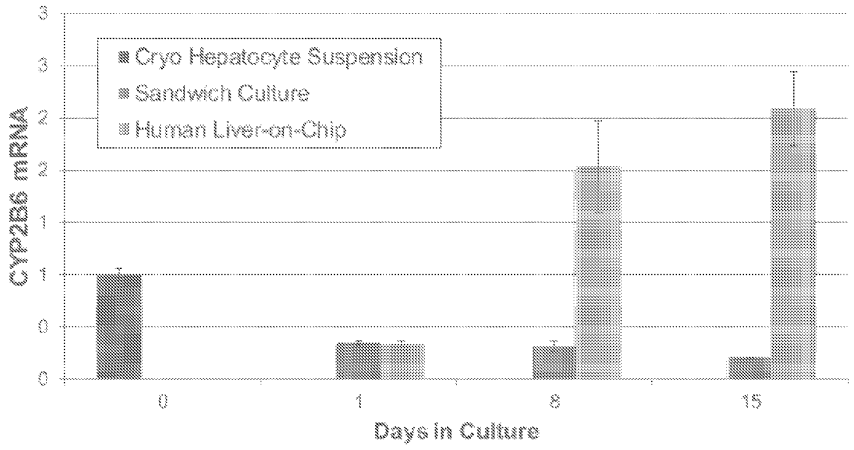

Cryo Hepatocyte Suspension
Sandwich Culture
Human Liver-on-Chip
CYP2B6 mRNA
Days in Culture
0
1
8
15

Figure 73

Figure 74

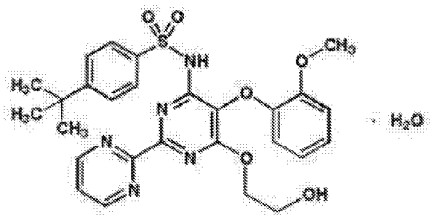


Figure 75

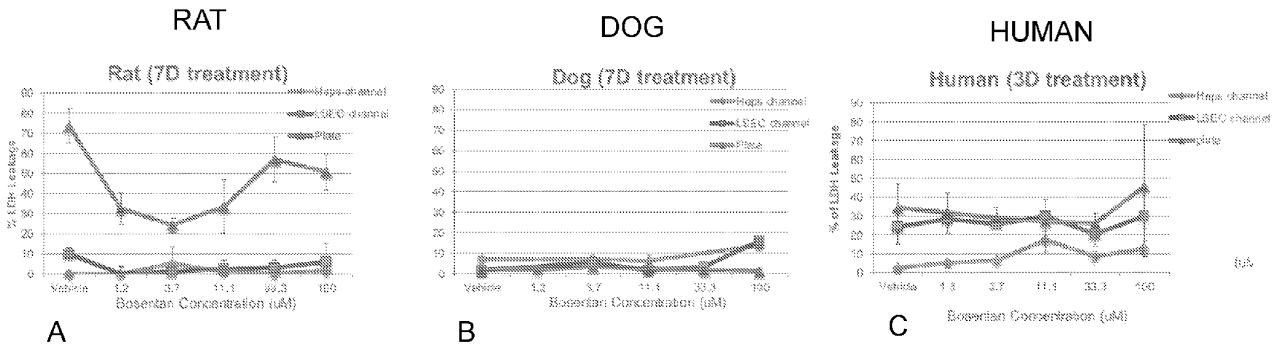
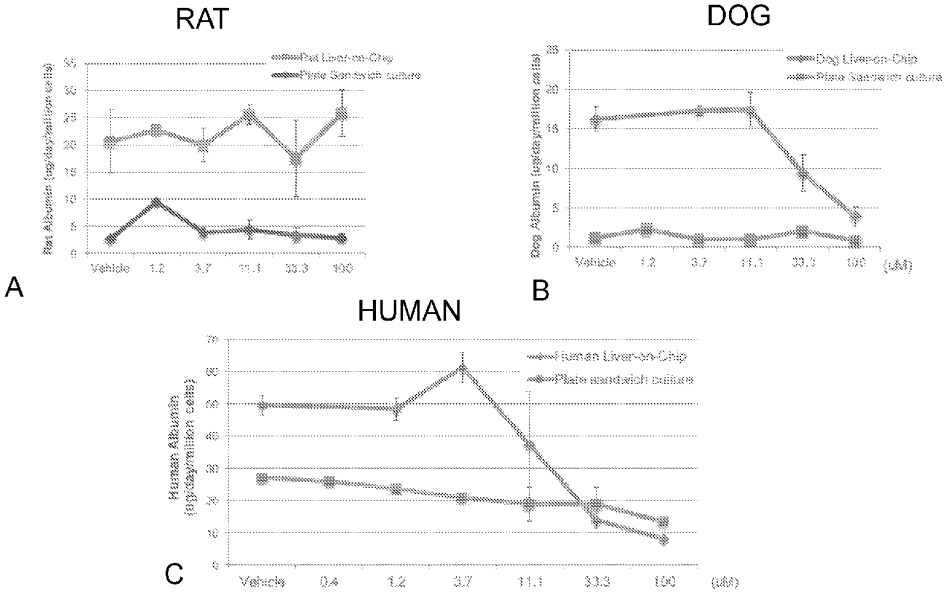

RAT
DOG
HUMAN
Rat (7D treatment)
Dog (7D treatment)
Human (3D treatment)
Hepa channel
LSEC channel
Plate
% LDH Leakage
Vehicle
1.2
3.7
11.1
33.3
100
Bosentan Concentration (uM)
A
B
C

Figure 76


RAT
Rat Liver-on-Chip
Plate Sandwich culture
Rat Albumin (ng/day/million cells)
Vehicle
1.2
3.7
11.1
33.3
100
A
DOG
Dog Liver-on-Chip
Plate Sandwich culture
Dog Albumin (ng/day/million cells)
Vehicle
1.2
3.7
11.1
33.3
100
(uM)
B
HUMAN
Human Liver-on-Chip
Plate sandwich culture
Human Albumin (ng/day/million cells)
Vehicle
0.4
1.2
3.7
11.1
33.3
100
(uM)
C

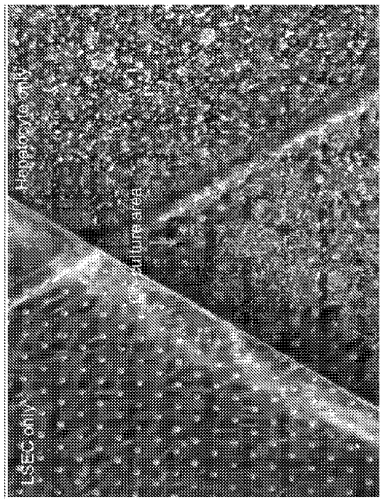
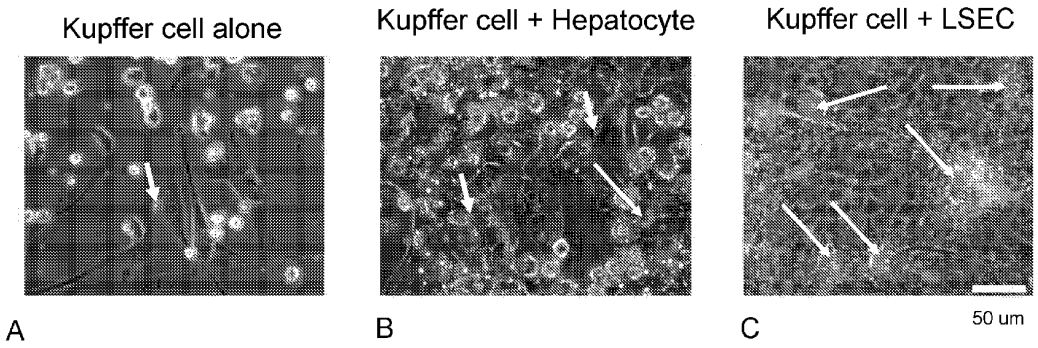


Figure 77

Figure 78


Kupffer cell alone
Kupffer cell + Hepatocyte
Kupffer cell + LSEC
A
B
C
50 um

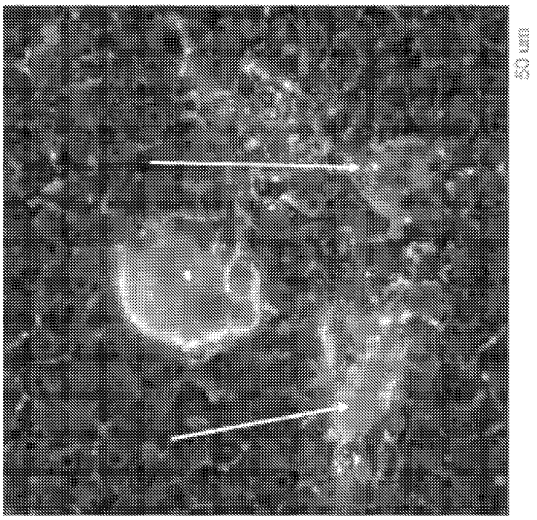


Figure 79

Figure 80

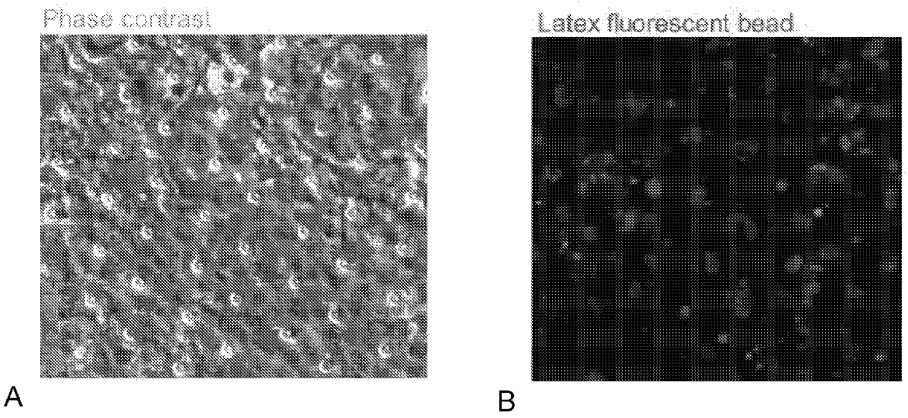
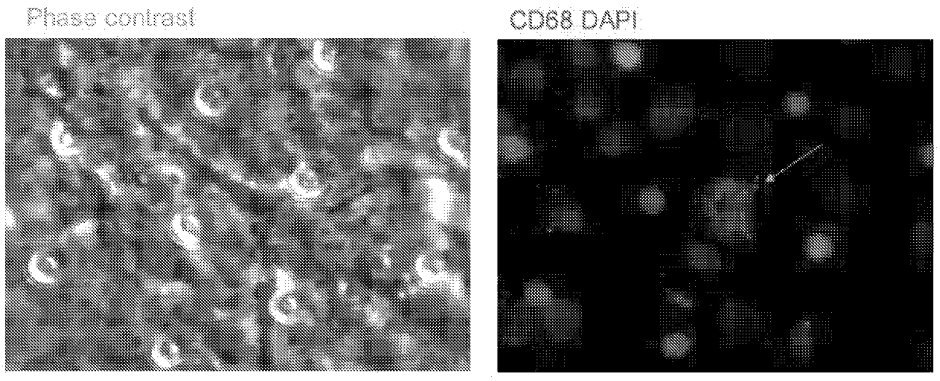


Figure 81



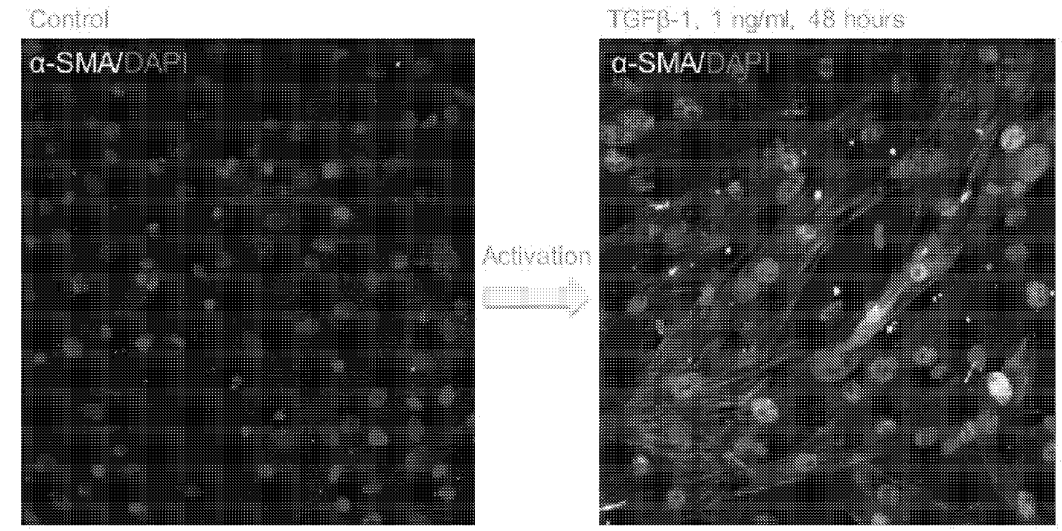

Control
α-SMA/DAPI
Activation
TGFβ-1, 1 ng/ml, 48 hours
α-SMA/DAPI
A
B

Figure 82

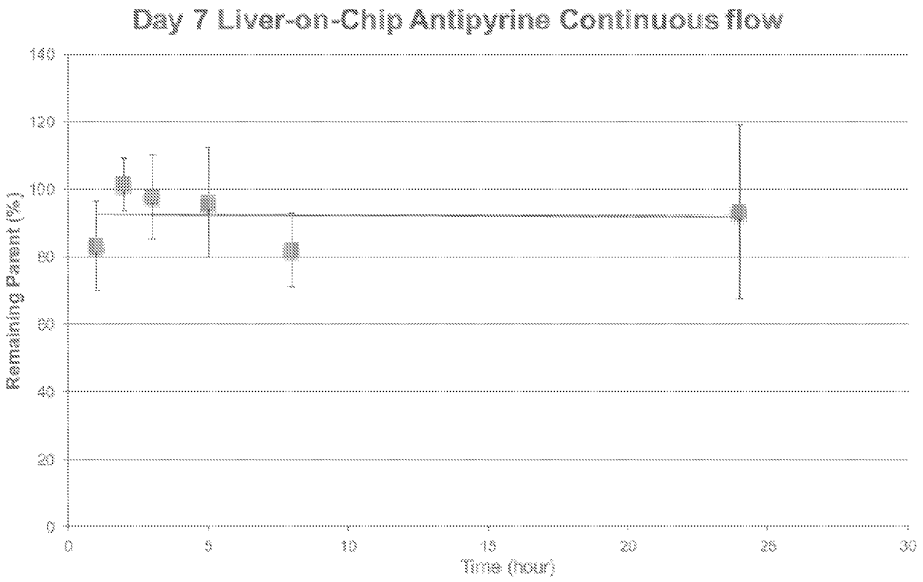

Day 7 Liver-on-Chip Antipyrine Continuous flow
Remaining Parent (%)
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (hour)

Figure 83

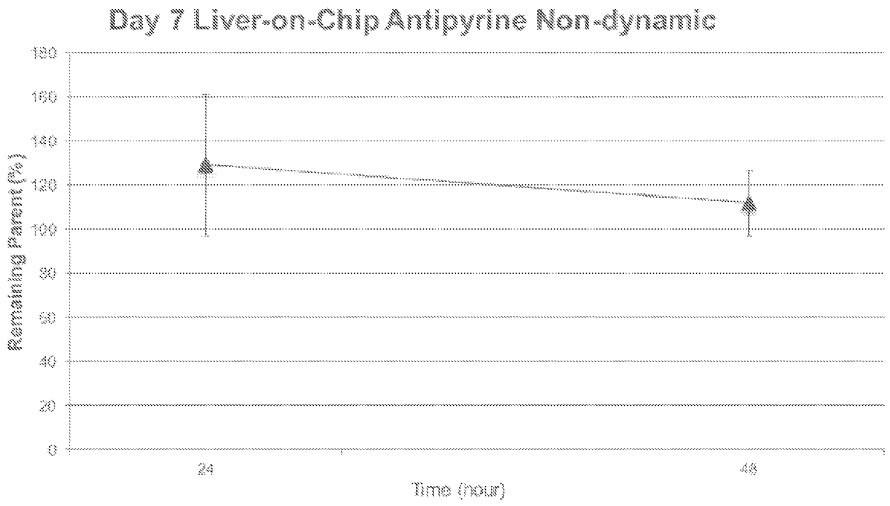

Day 7 Liver-on-Chip Antipyrine Non-dynamic
Remaining Parent (%)
180
160
140
120
100
80
60
40
20
0
24
48
Time (hour)

Figure 84

Figure 85

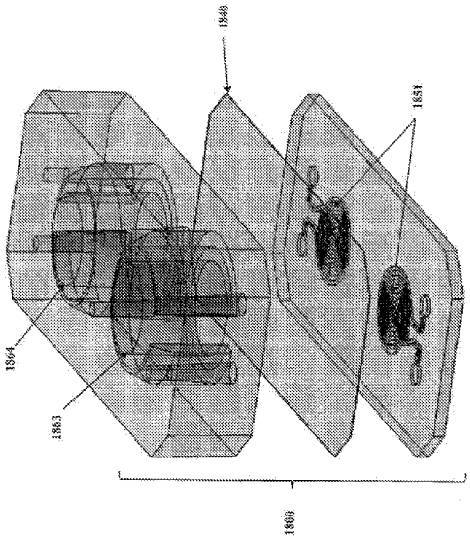


Figure 86

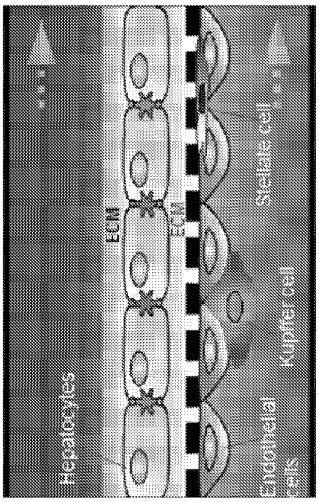
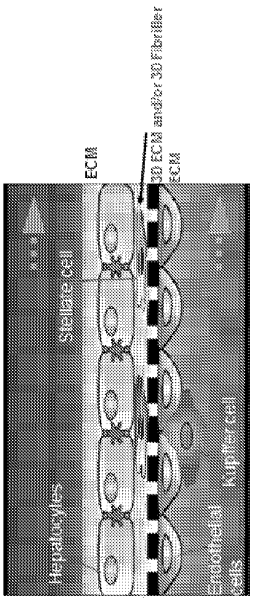

Hepatocytes
ECM
ECM
Stellate cell
Endothelial cells
Kupffer cell

Figure 87


Hepatocytes
Stellate cell
ECM
3D ECM and/or 3D Fibrillar ECM
Endothelial cells
Kupffer cell

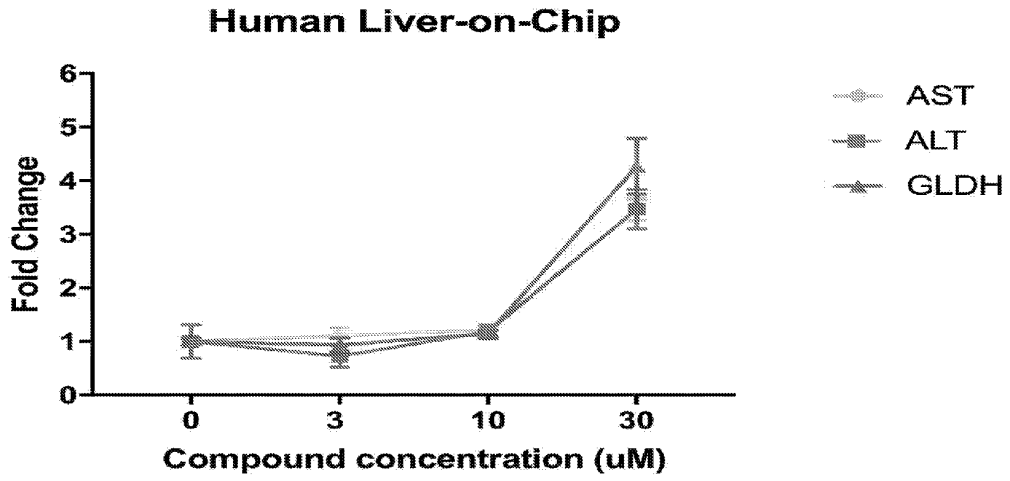

Human Liver-on-Chip
Fold Change
0
1
2
3
4
5
6
0
3
10
30
Compound concentration (uM)
AST
ALT
GLDH

Figure 88

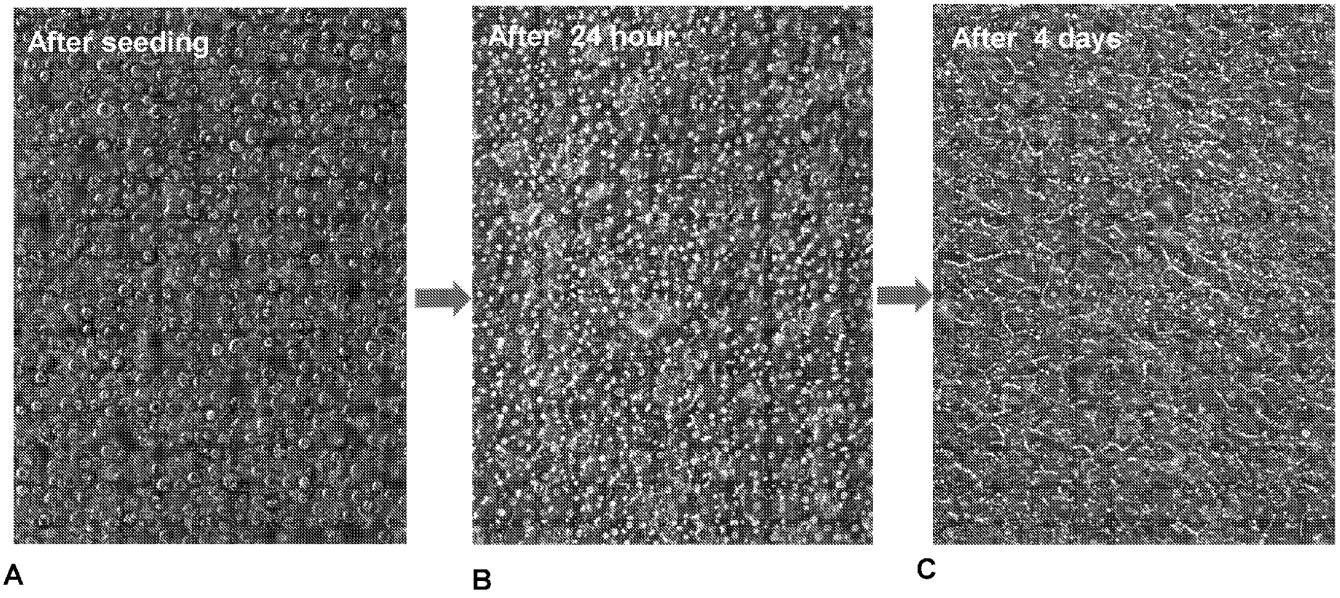


Figure 89

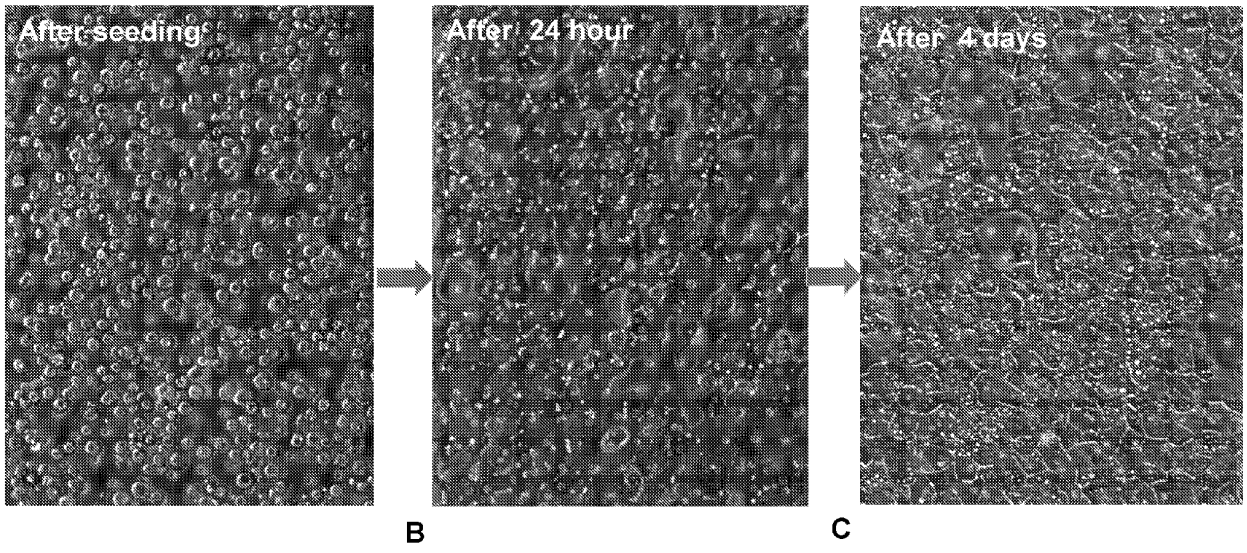


Figure 90

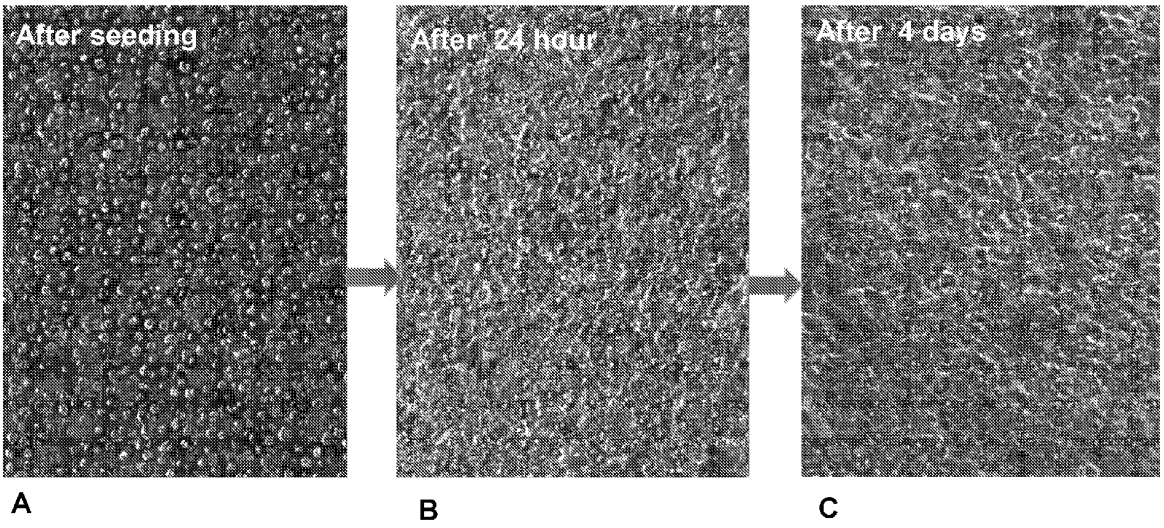


Figure 91