FIGURE 1A

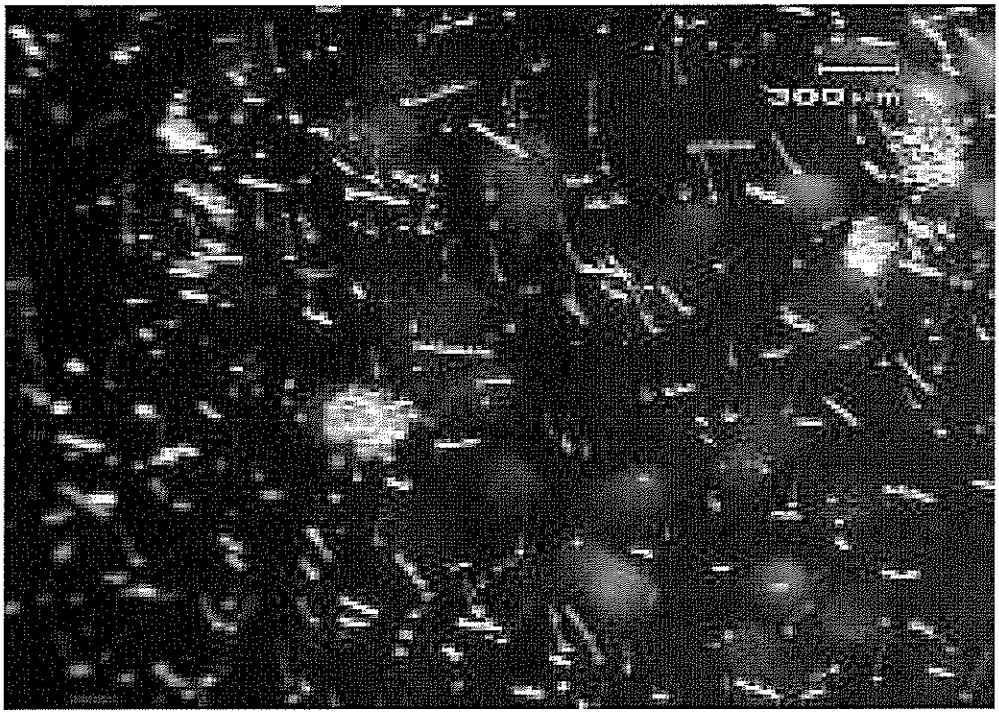


FIGURE 1B

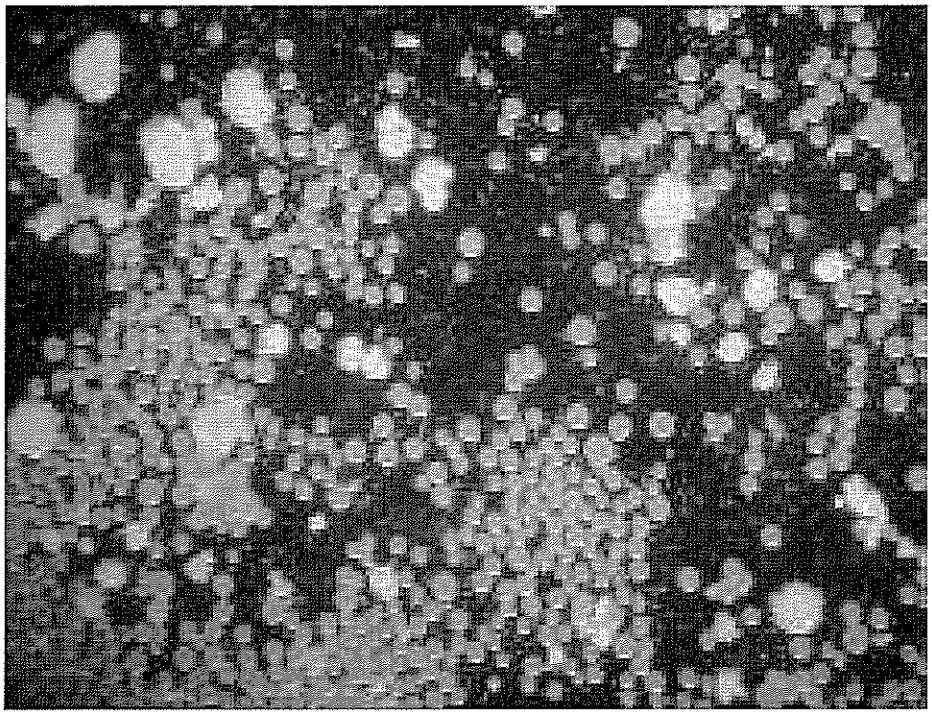


FIGURE 1C

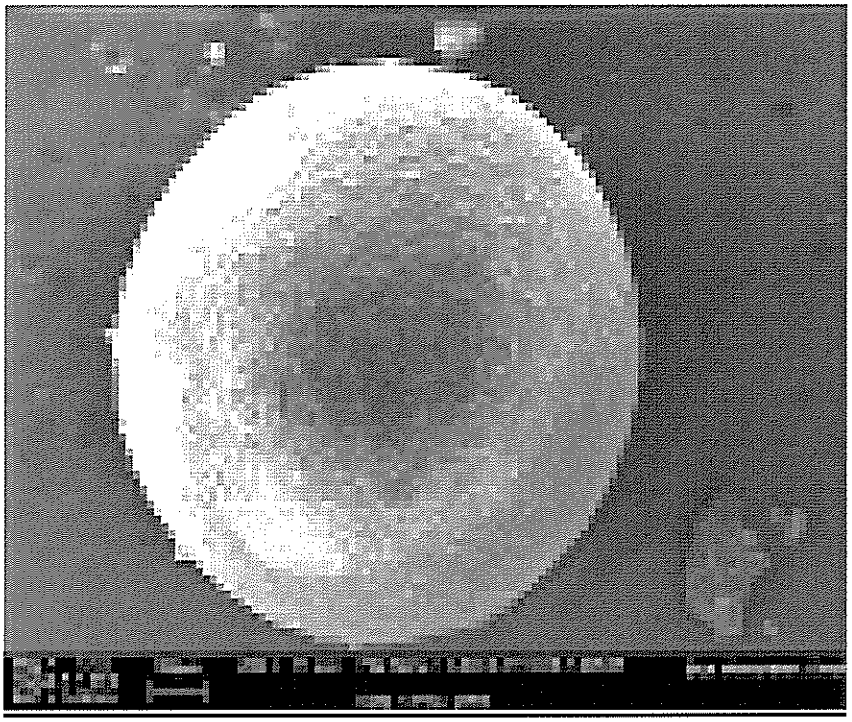


FIGURE 1D

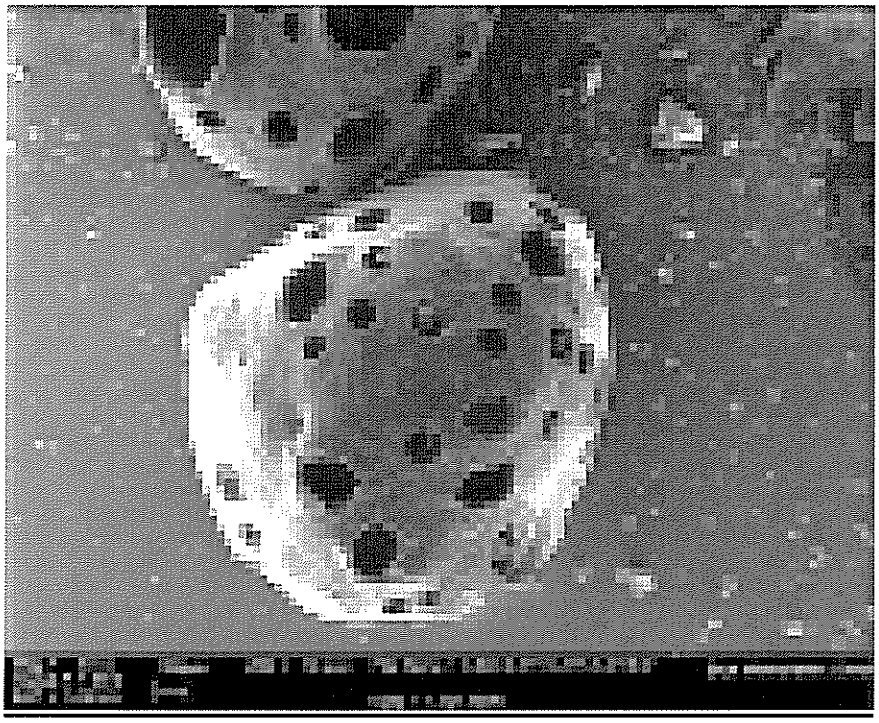


FIGURE 2

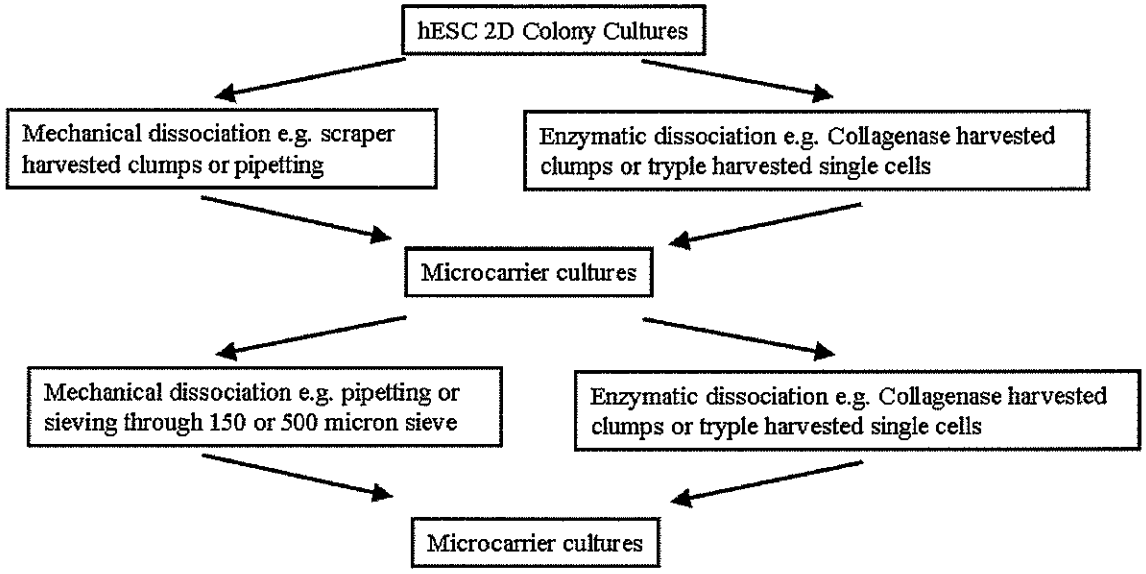

hESC 2D Colony Cultures
Mechanical dissociation e.g. scraper harvested clumps or pipetting
Enzymatic dissociation e.g. Collagenase harvested clumps or tryple harvested single cells
Microcarrier cultures
Mechanical dissociation e.g. pipetting or sieving through 150 or 500 micron sieve
Enzymatic dissociation e.g. Collagenase harvested clumps or tryple harvested single cells
Microcarrier cultures

FIGURE 3

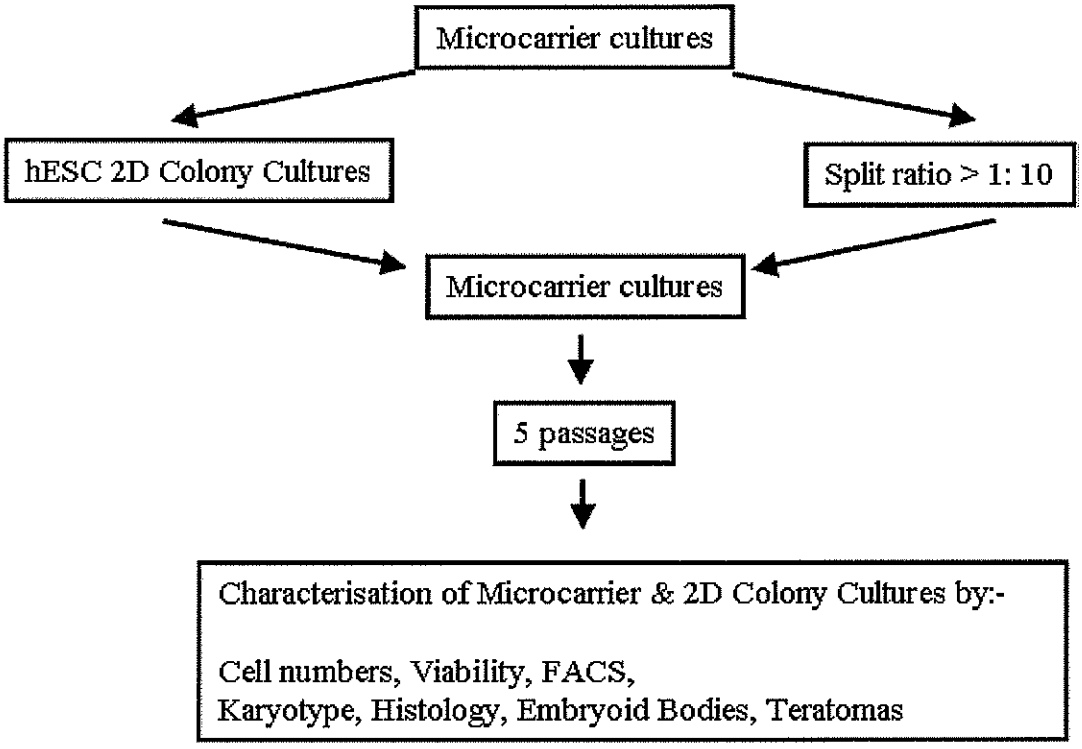

Microcarrier cultures
hESC 2D Colony Cultures
Split ratio > 1: 10
Microcarrier cultures
5 passages
Characterisation of Microcarrier & 2D Colony Cultures by:-
Cell numbers, Viability, FACS,
Karyotype, Histology, Embryoid Bodies, Teratomas

FIGURE 4

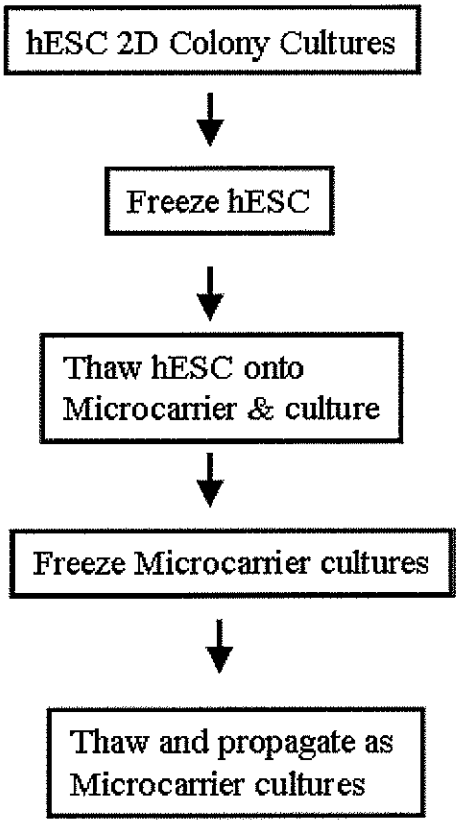
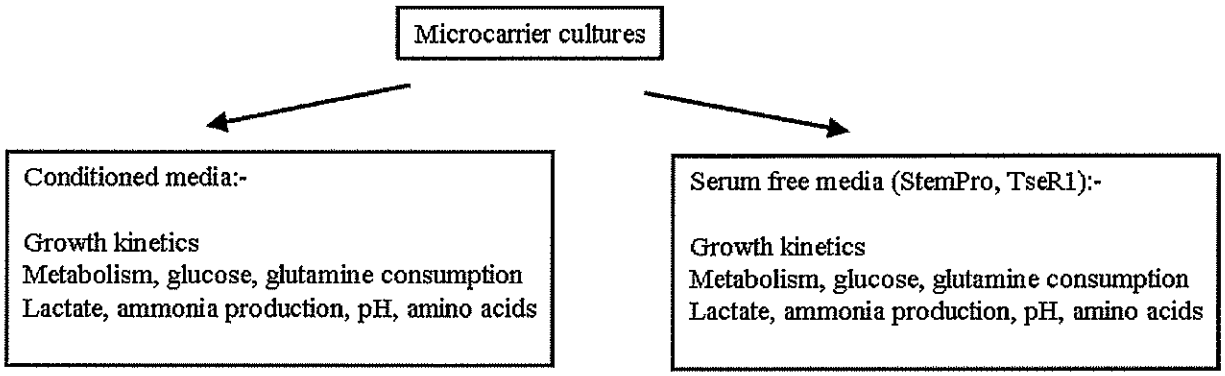

hESC 2D Colony Cultures
Freeze hESC
Thaw hESC onto Microcarrier & culture
Freeze Microcarrier cultures
Thaw and propagate as Microcarrier cultures

FIGURE 5



Grown for 4 passages in serum free media on microcarriers

FIGURE 6A

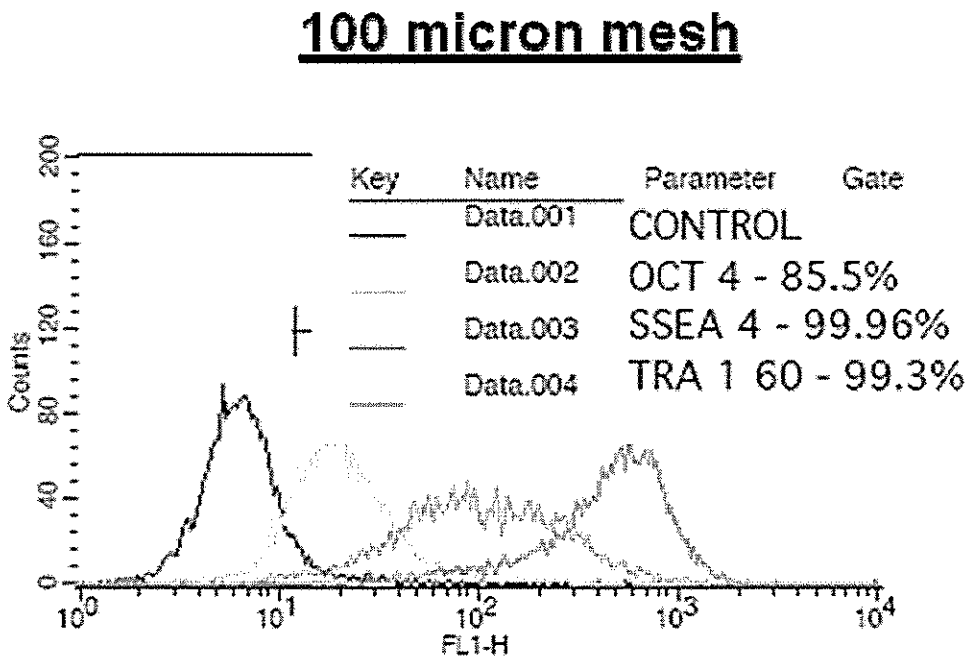

100 micron mesh
Key
Name
Parameter
Gate
Data.001
CONTROL
Data.002
OCT 4 - 85.5%
Data.003
SSEA 4 - 99.96%
Data.004
TRA 1 60 - 99.3%
Counts
0
40
80
120
160
200
10^0
10^1
10^2
10^3
10^4
FL1-H

FIGURE 6B

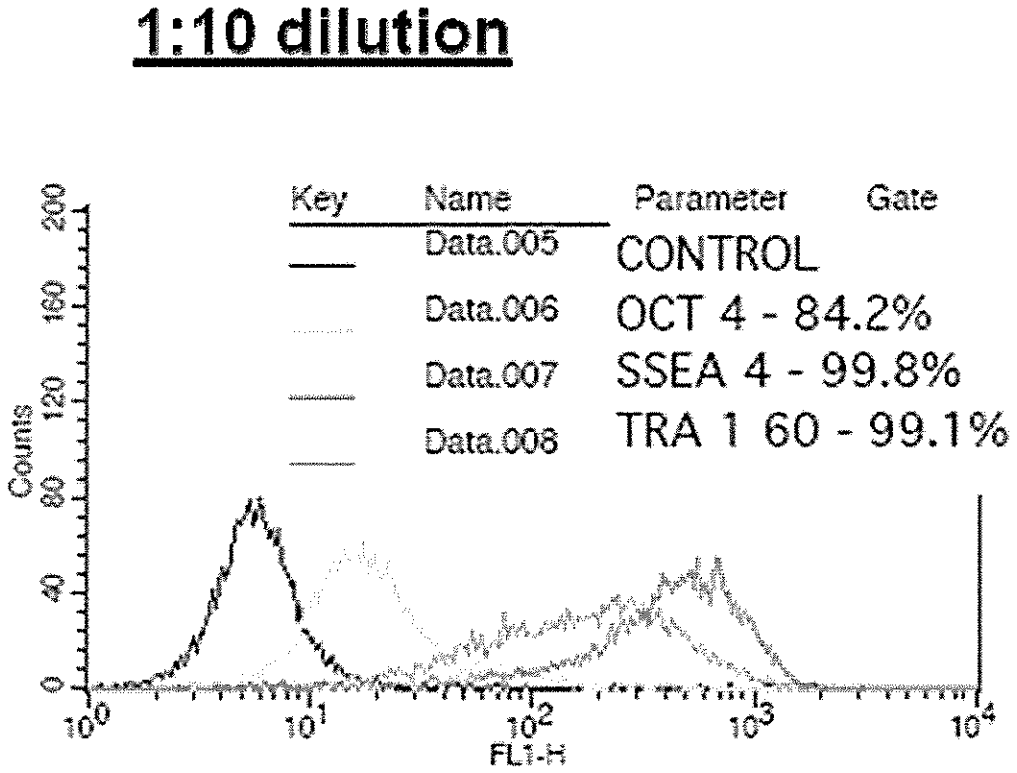

1:10 dilution
Key
Name
Parameter
Gate
Data.005
CONTROL
Data.006
OCT 4 - 84.2%
Data.007
SSEA 4 - 99.8%
Data.008
TRA 1 60 - 99.1%
Counts
0
40
80
120
160
200
10^0
10^1
10^2
10^3
10^4
FL1-H

FIGURE 6C

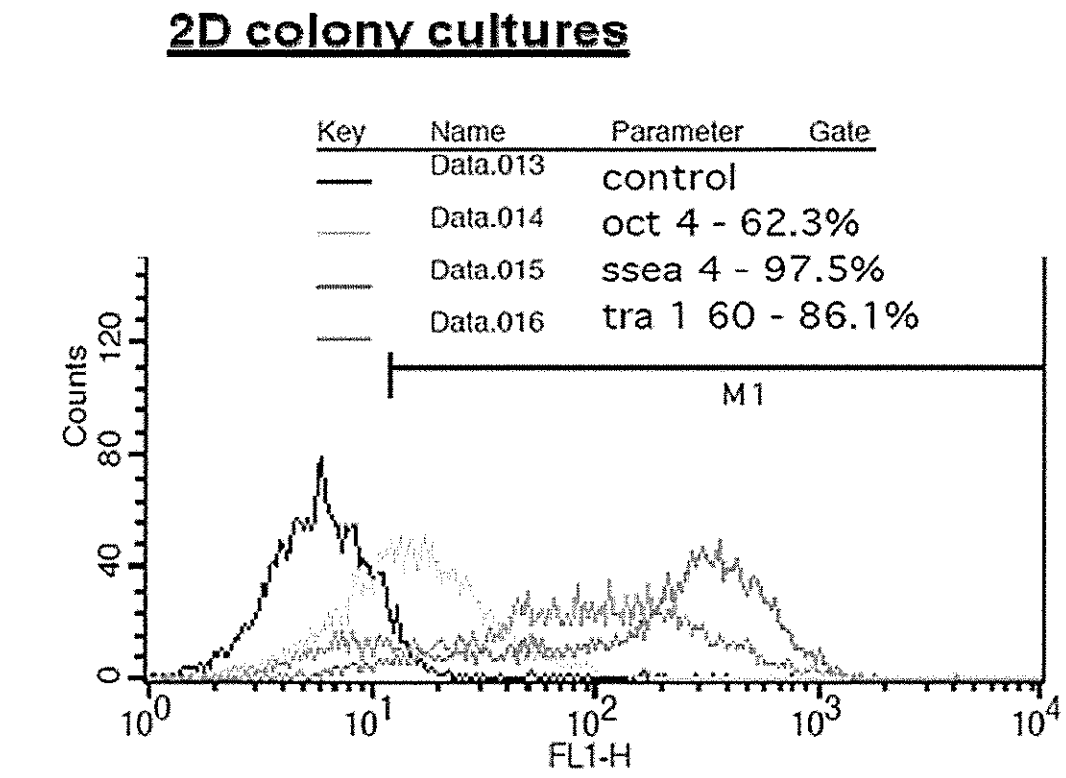

2D colony cultures
Key
Name
Parameter
Gate
Data.013
control
Data.014
oct 4 - 62.3%
Data.015
ssea 4 - 97.5%
Data.016
tra 1 60 - 86.1%
M1
Counts
0
40
80
120
10^0
10^1
10^2
10^3
10^4
FL1-H

FIGURE 6D

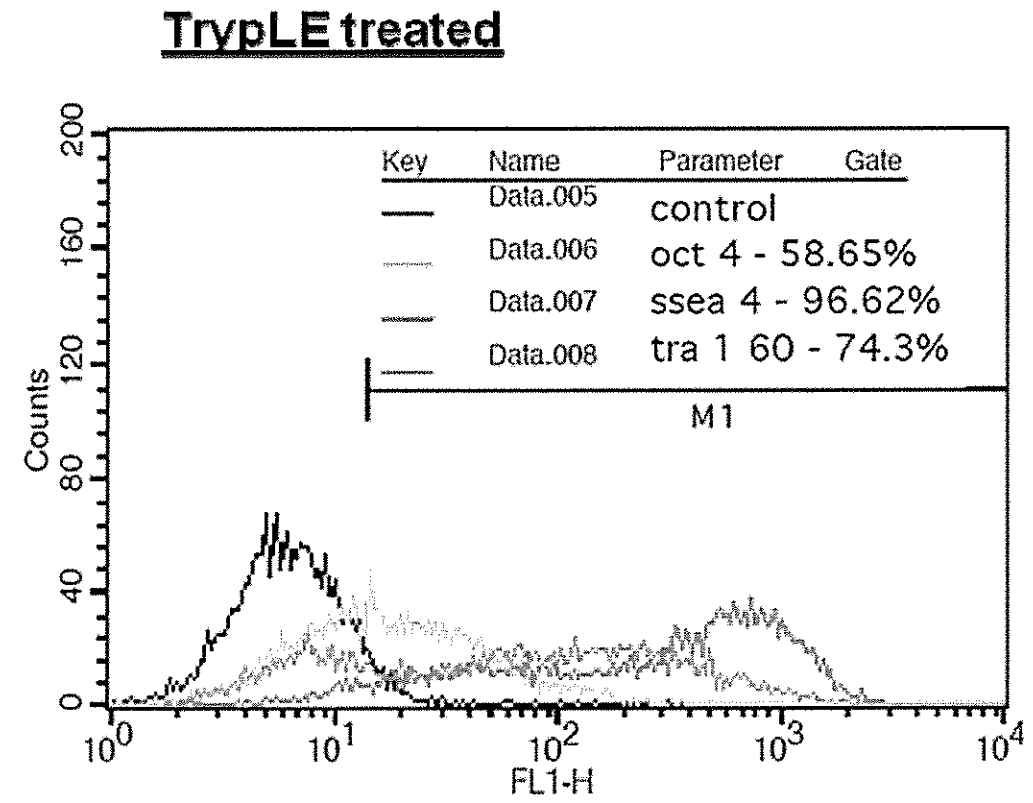

TrypLE treated
Key
Name
Parameter
Gate
Data.005
control
Data.006
oct 4 - 58.65%
Data.007
ssea 4 - 96.62%
Data.008
tra 1 60 - 74.3%
M1
Counts
0
40
80
120
160
200
10^0
10^1
10^2
10^3
10^4
FL1-H

FIGURE 7A

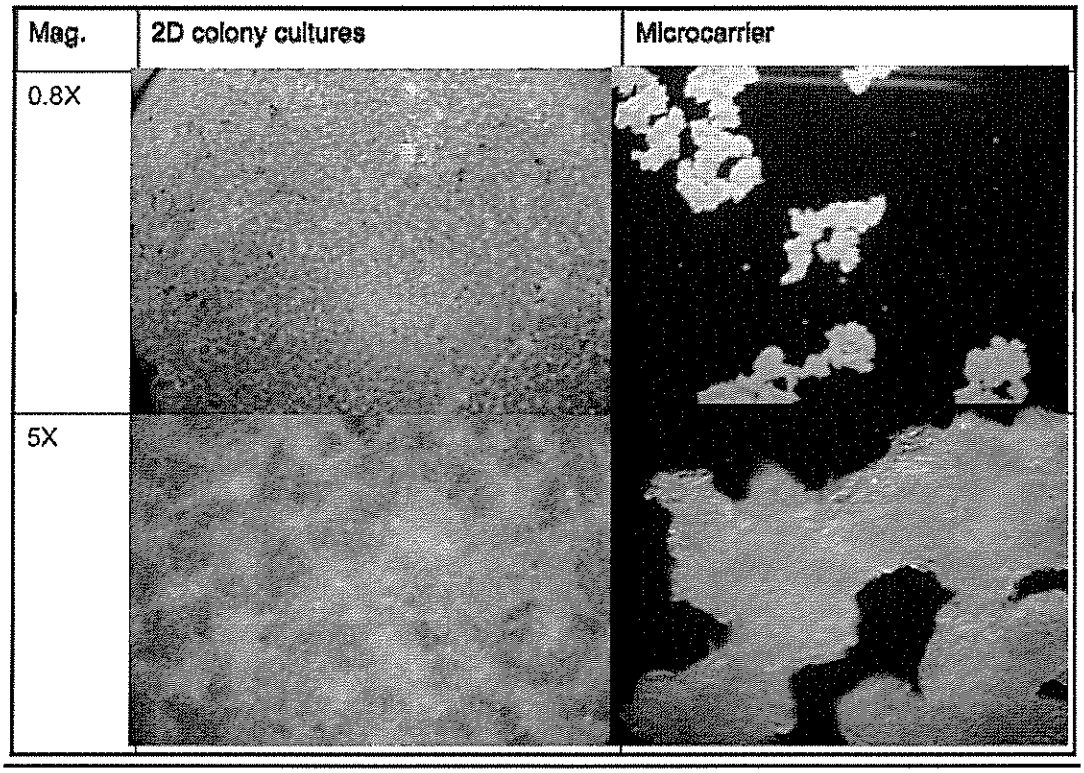


FIGURE 7B

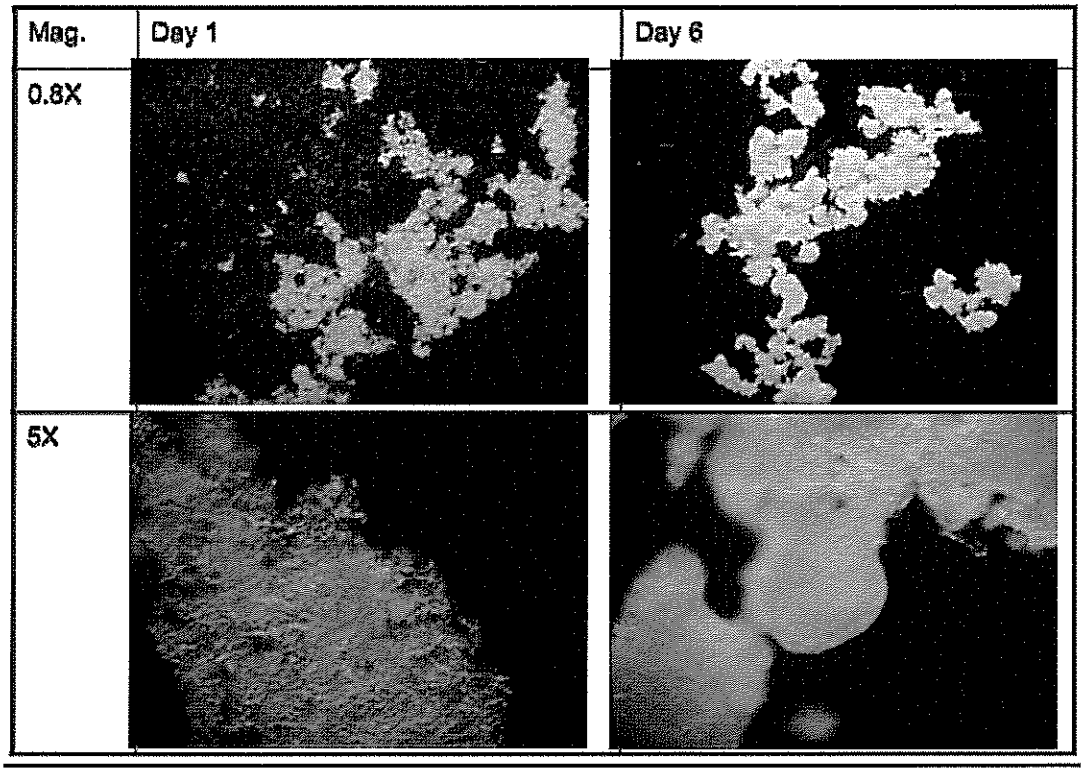
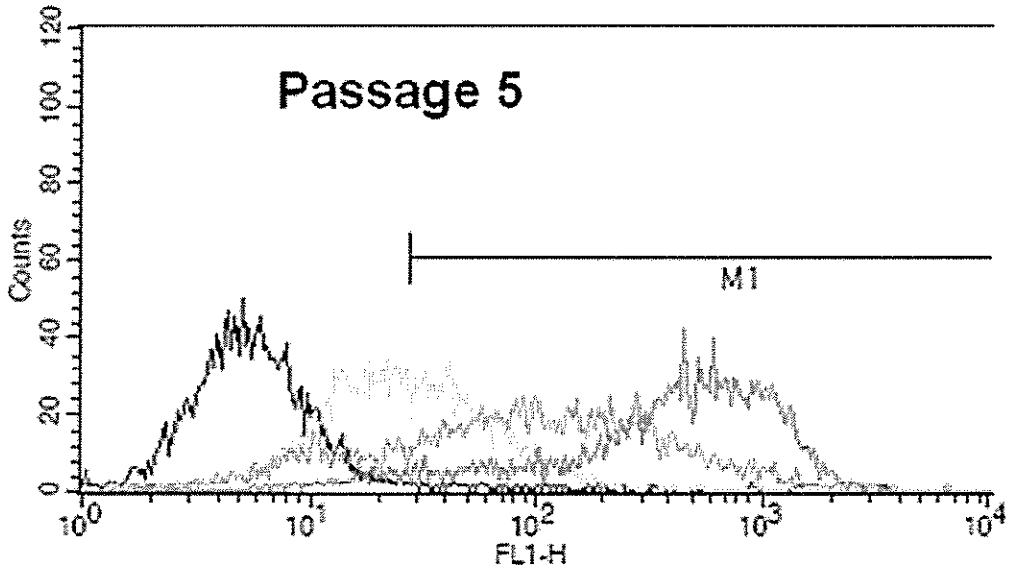


FIGURE 8A



Key	Name	Parameter	Gate
——	CONTROL		
	OCT 4 - 80.2%		
___	SSEA 4 - 99.3%		
___	TRA 1 60 - 88.6%		

FIGURE 8B

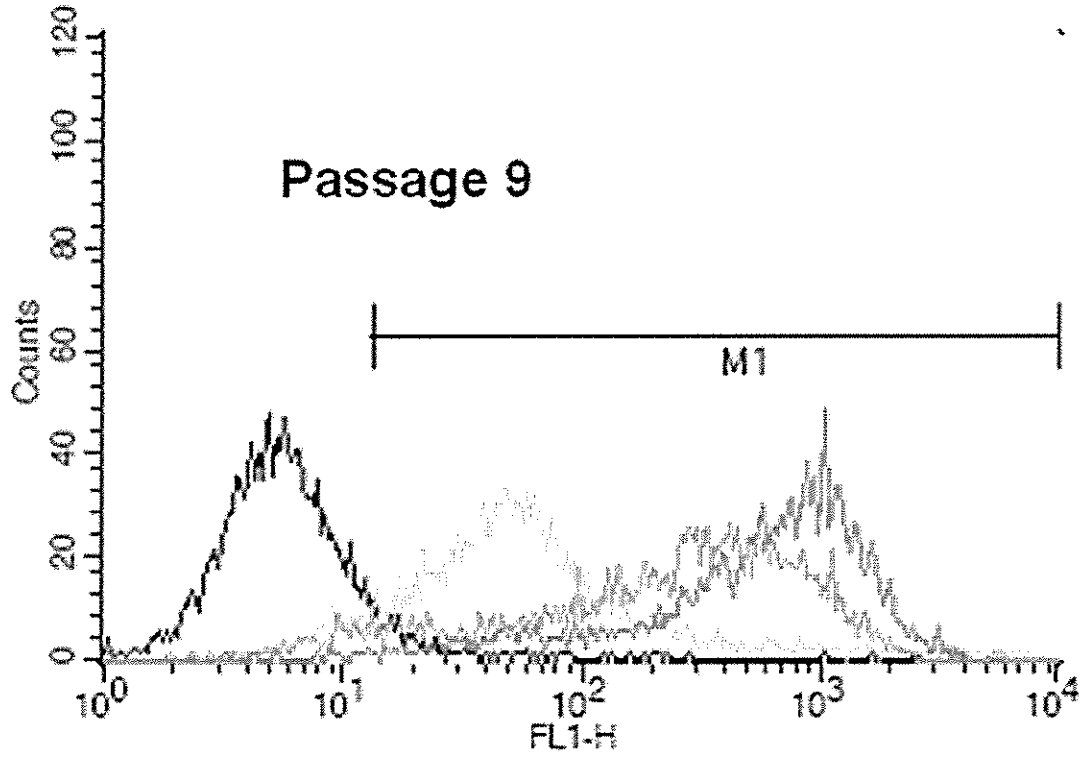

Passage 9
M1
Counts
0
20
40
60
80
100
120
10^0
10^1
10^2
10^3
10^4
FL1-H

FIGURE 8C

-

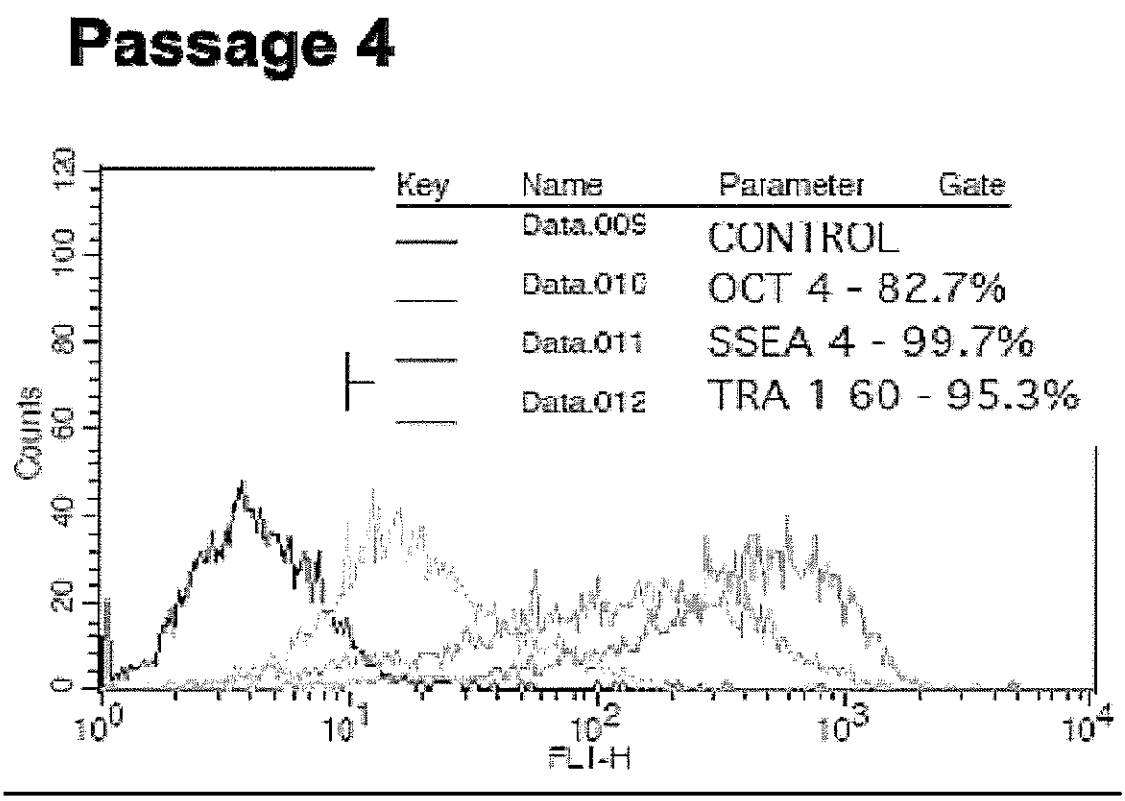

Passage 4
Key
Name
Parameter
Gate
Data.009
CONTROL
Data.010
OCT 4 - 82.7%
Data.011
SSEA 4 - 99.7%
Data.012
TRA 1 60 - 95.3%
Counts
FL1-H

FIGURE 8D AND FIGURE 8E

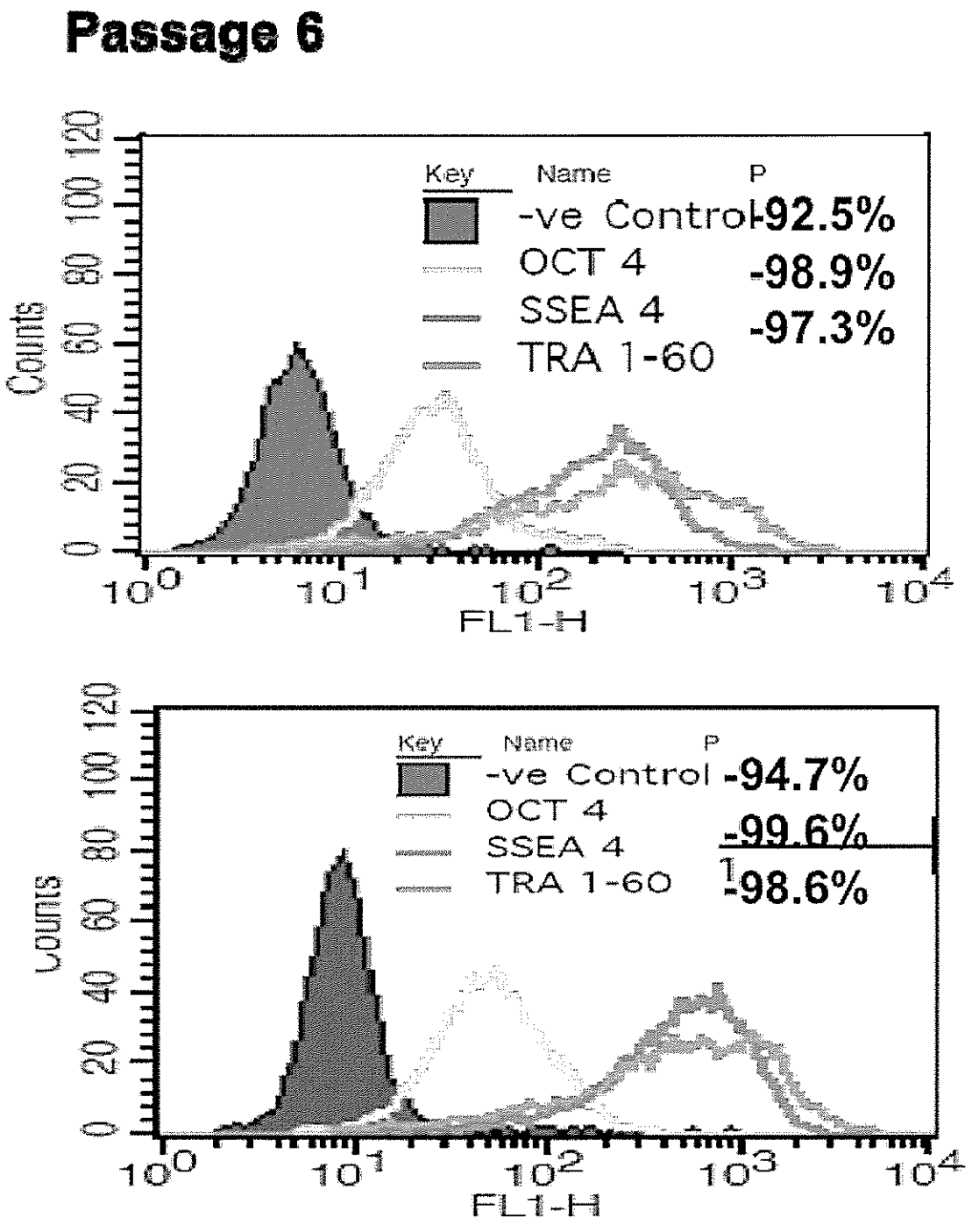


FIGURE 9

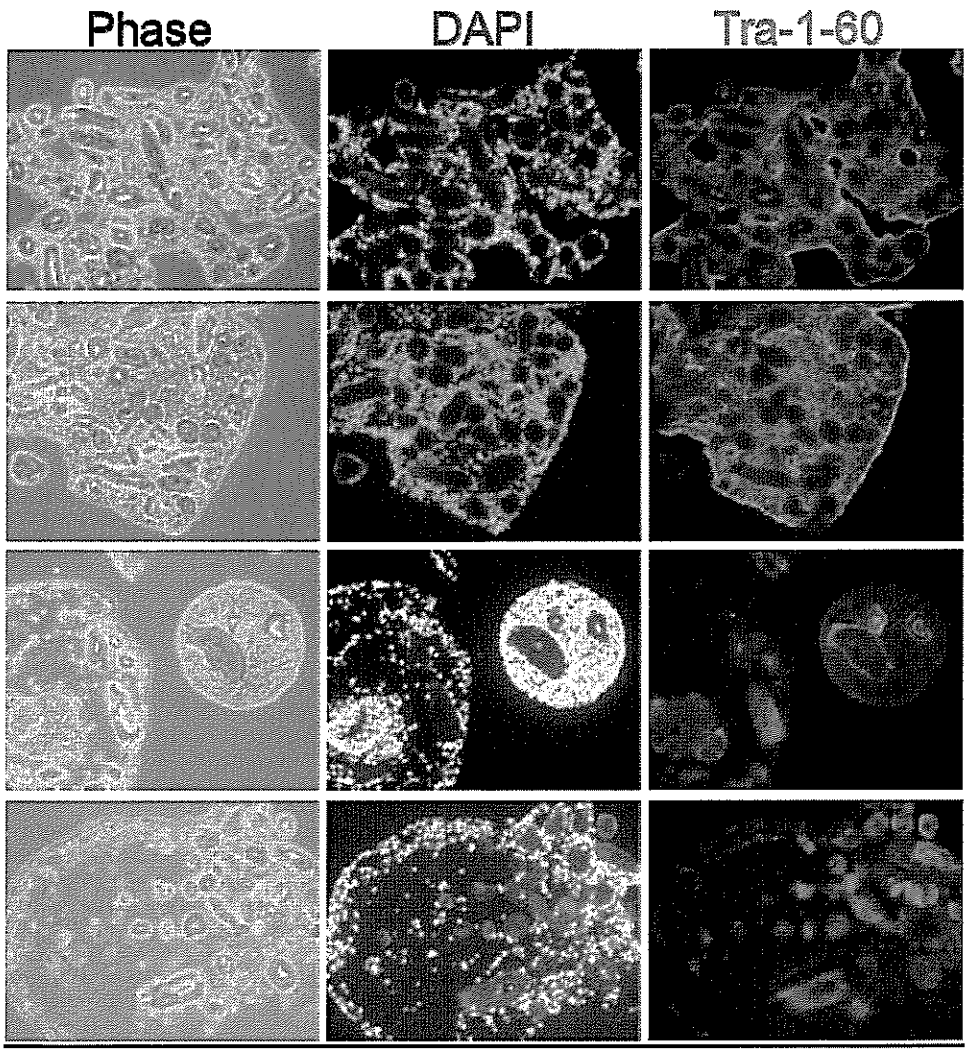


FIGURE 10A

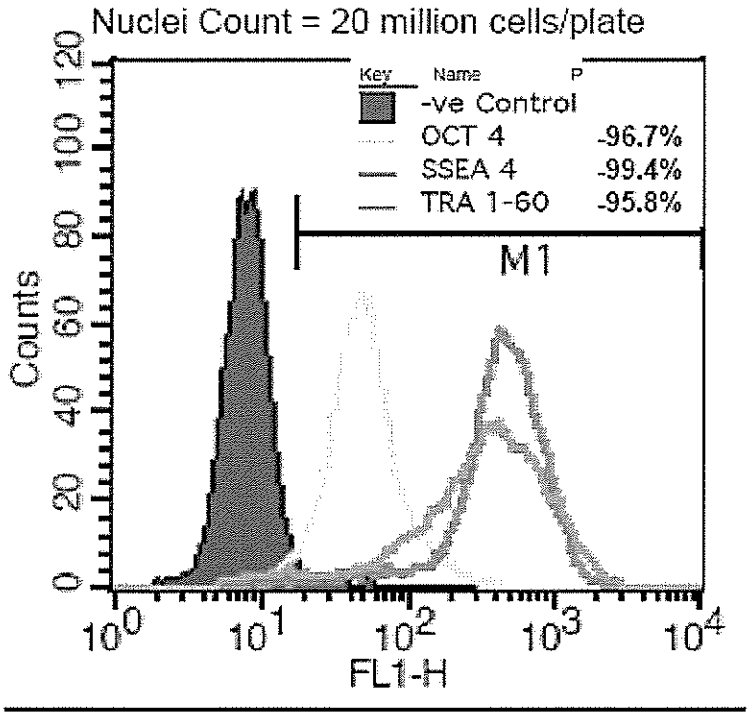

Nuclei Count = 20 million cells/plate
Key
Name
P
-ve Control
OCT 4
-96.7%
SSEA 4
-99.4%
TRA 1-60
-95.8%
M1
Counts
0
20
40
60
80
100
120
10^0
10^1
10^2
10^3
10^4
FL1-H

FIGURE10B

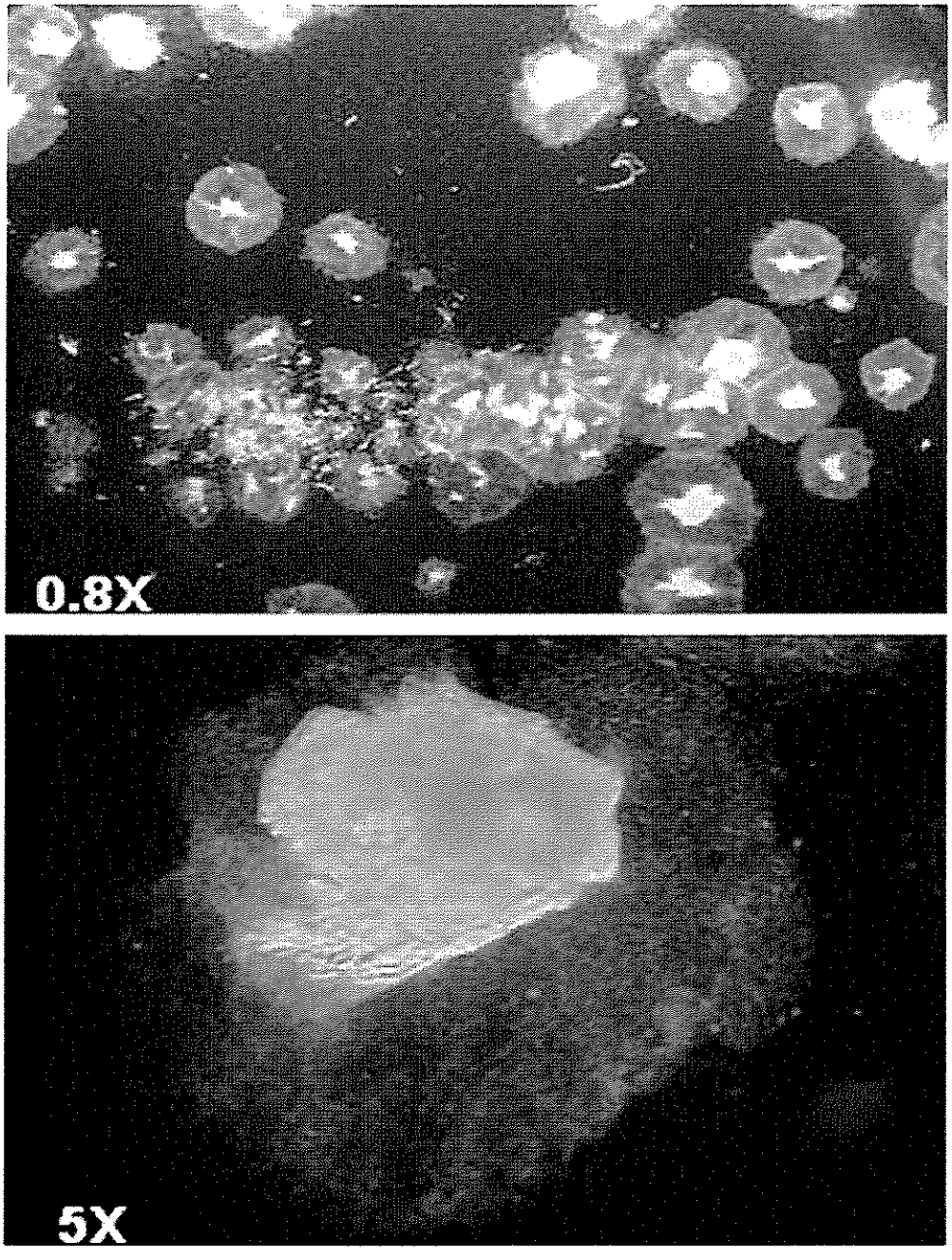
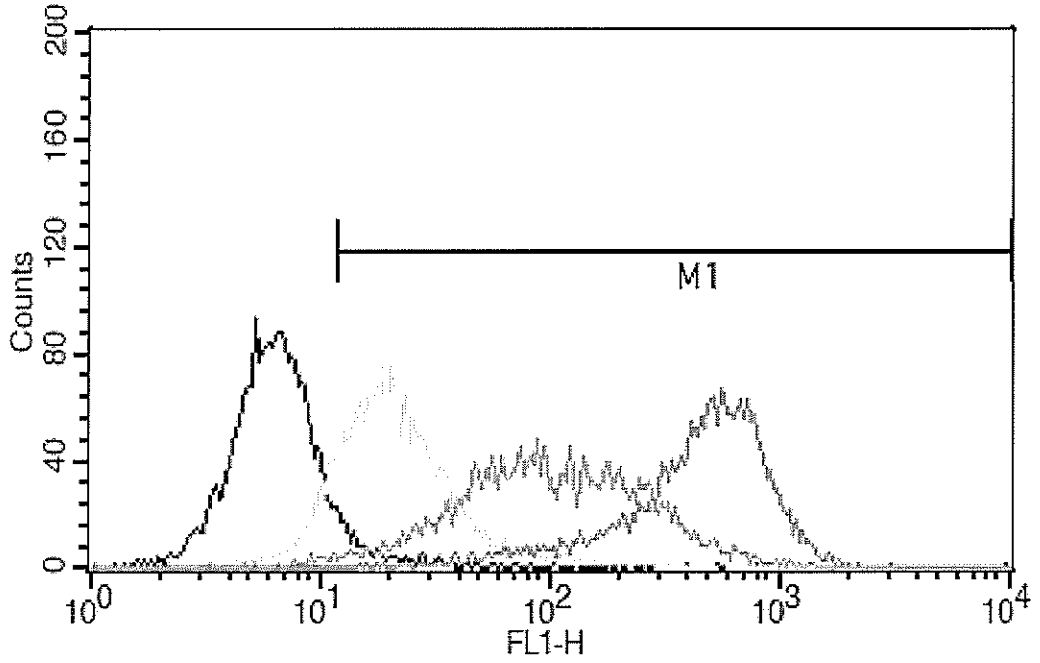


FIGURE 11A



Key	Name	Parameter	Gate
———	Data.001	CONTROL	
.....	Data.002	OCT 4 - 85.5%	
———	Data.003	SSEA 4 - 99.96%	
———	Data.004	TRA 1 60 - 99.3%	

FIGURE 11B

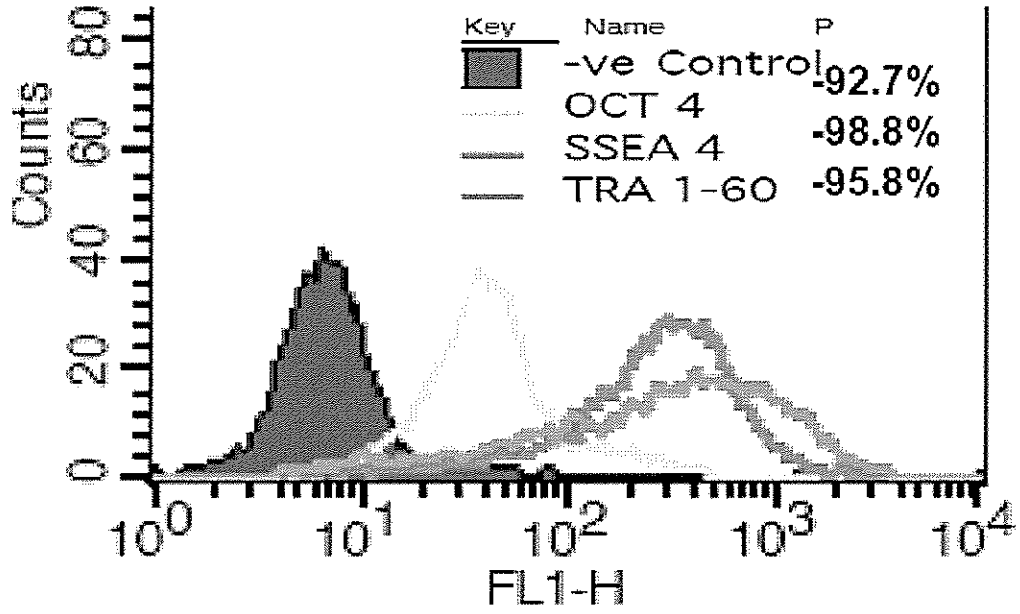

Key
Name
P
-ve Control
-92.7%
OCT 4
-98.8%
SSEA 4
-95.8%
TRA 1-60
Counts
0
20
40
60
80
10^0
10^1
10^2
10^3
10^4
FL1-H

FIGURE 12

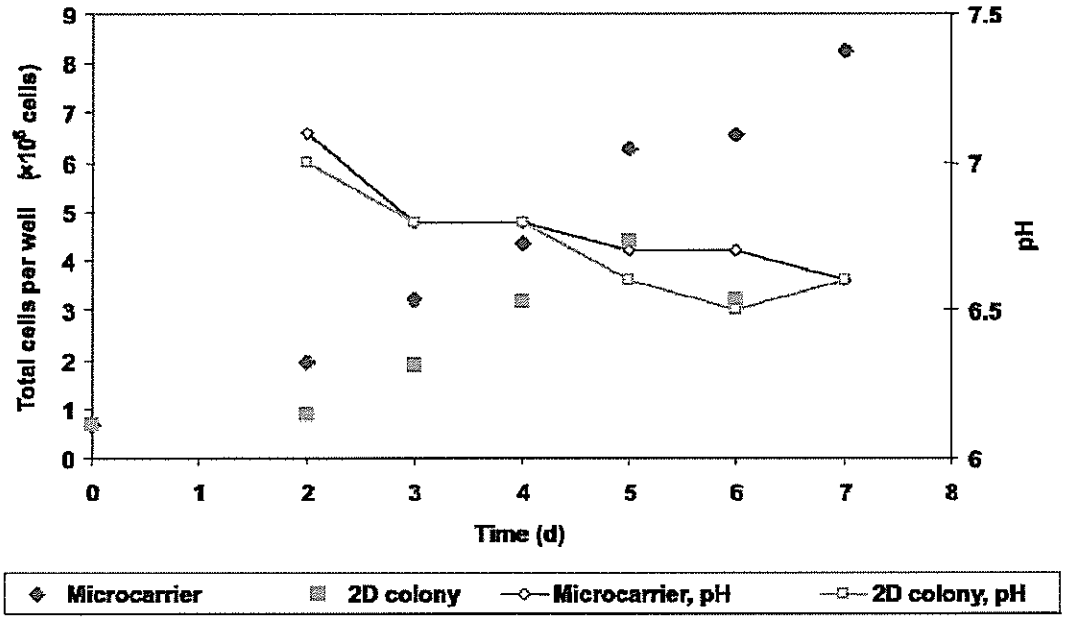

Total cells per well (x10^6 cells)
pH
Time (d)
0
1
2
3
4
5
6
7
8
9
6
6.5
7
7.5
Microcarrier
2D colony
Microcarrier, pH
2D colony, pH

FIGURE 13

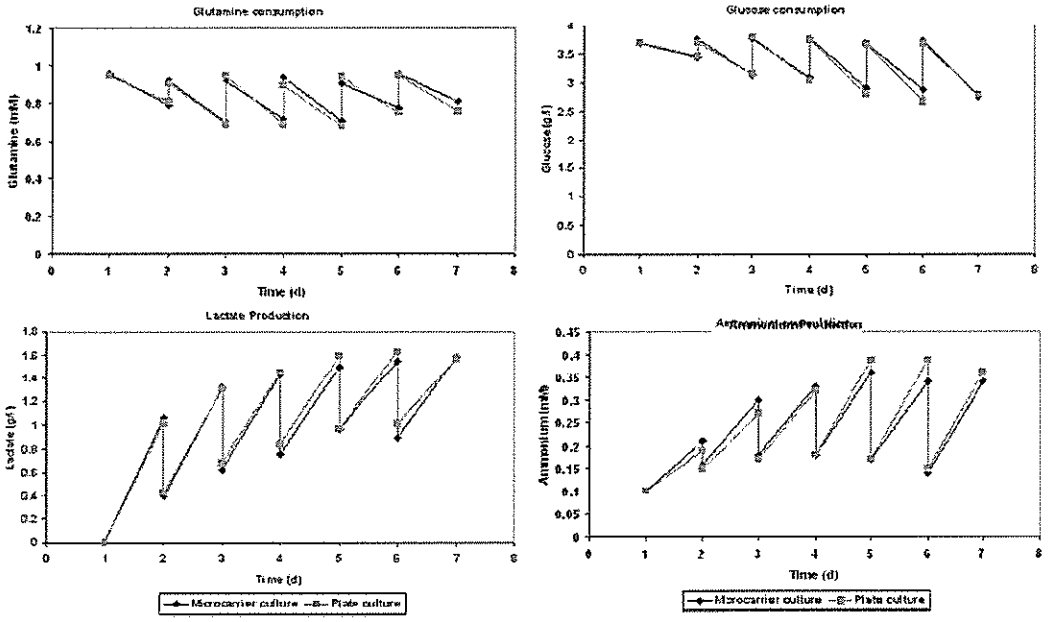

Glutamine consumption
Glutamine (mM)
Time (d)
Glucose consumption
Glucose (g/l)
Time (d)
Lactate Production
Lactate (g/l)
Time (d)
Ammonium Production
Ammonium (mM)
Time (d)
Microcarrier culture
Plate culture

FIGURE 14

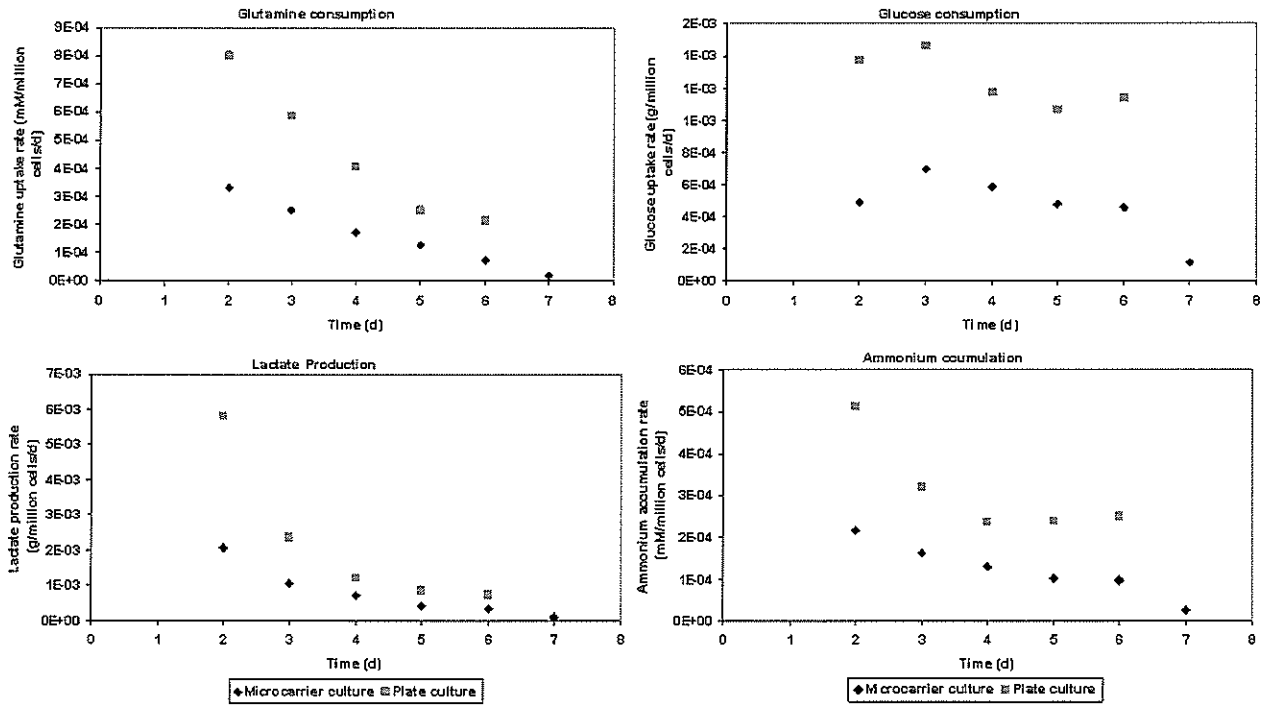

Glutamine consumption
Glutamine uptake rate (mM/million cells/d)
Time (d)
Glucose consumption
Glucose uptake rate (g/million cells/d)
Time (d)
Lactate Production
Lactate production rate (g/million cells/d)
Time (d)
Microcarrier culture
Plate culture
Ammonium coumulation
Ammonium accumulation rate (mM/million cells/d)
Time (d)
Microcarrier culture
Plate culture

FIGURE 15

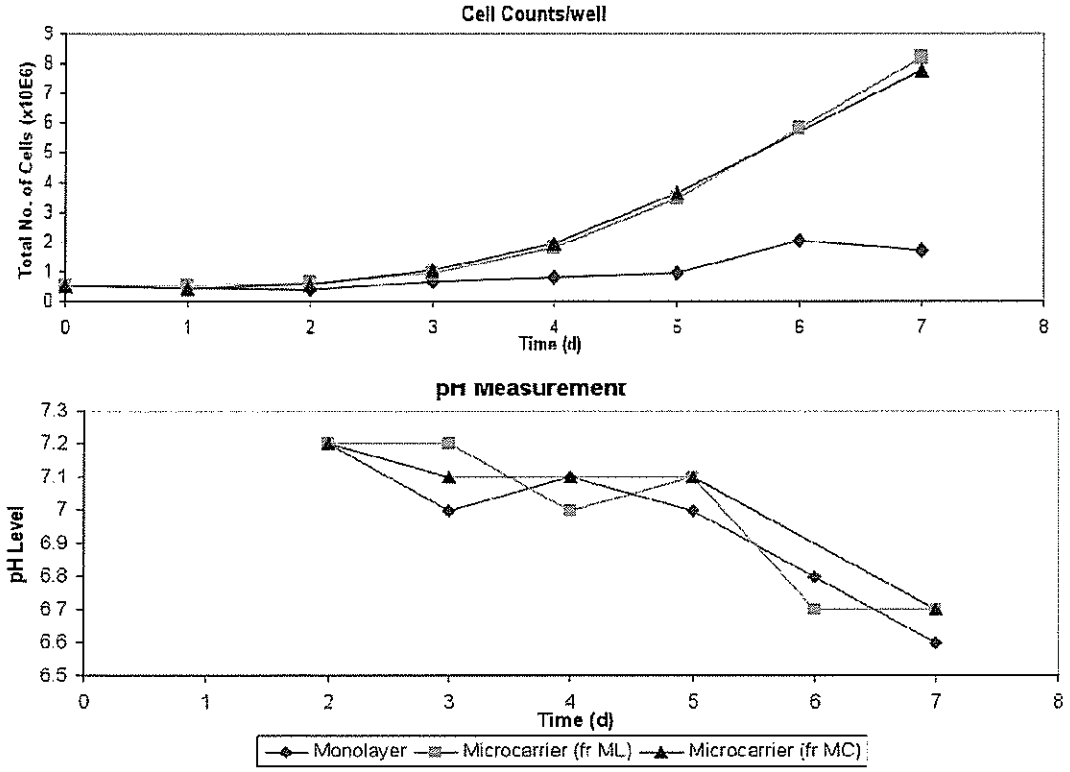
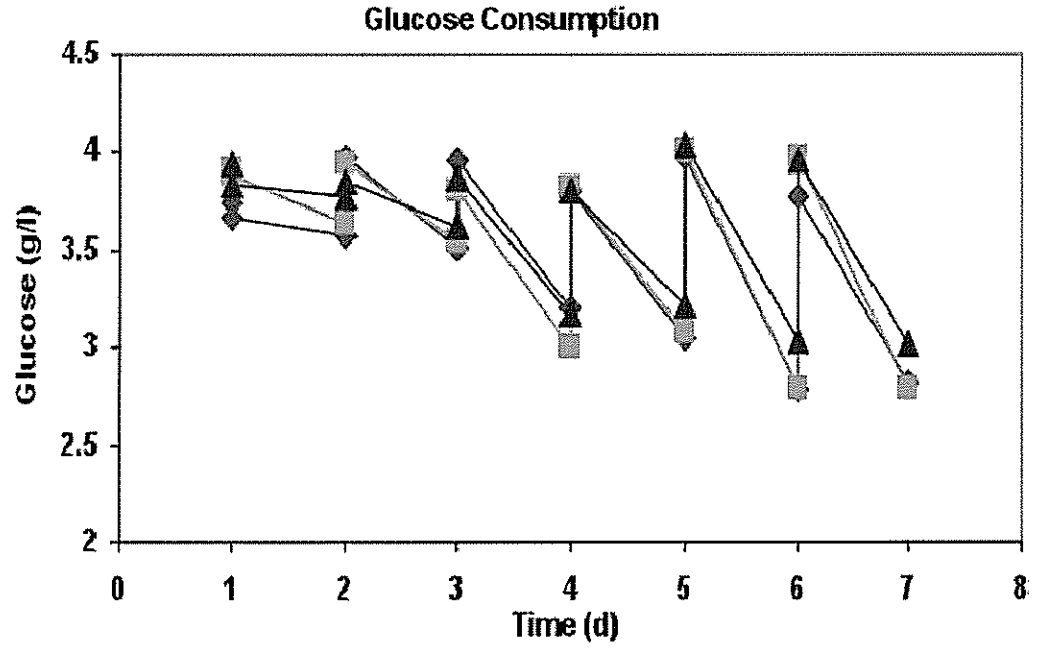

Cell Counts/well
Total No. of Cells (x10E6)
Time (d)
pH Measurement
pH Level
Time (d)
Monolayer
Microcarrier (fr ML)
Microcarrier (fr MC)

FIGURE 16


Glucose Consumption
Glucose (g/l)
4.5
4
3.5
3
2.5
2
0
1
2
3
4
5
6
7
8
Time (d)

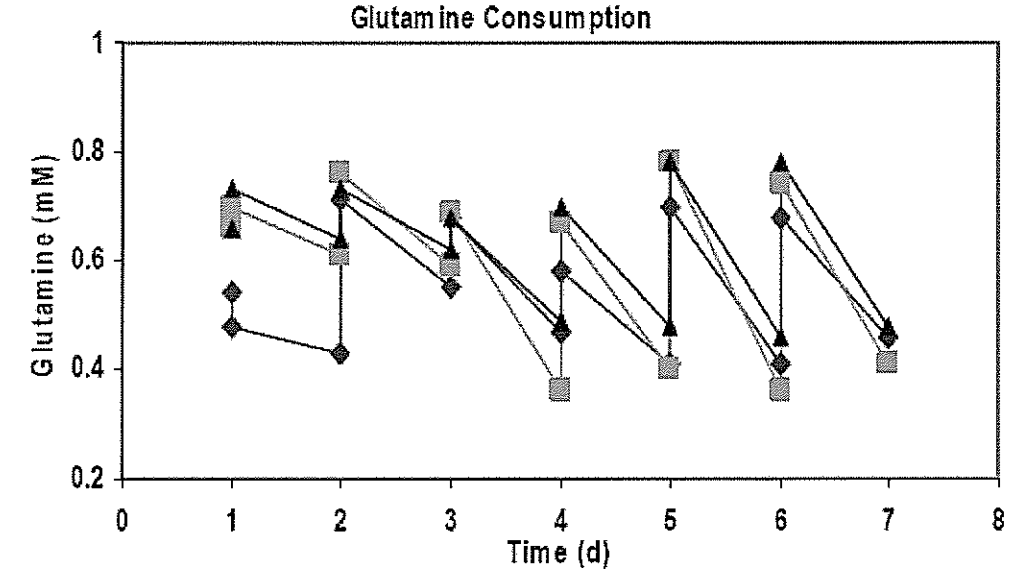
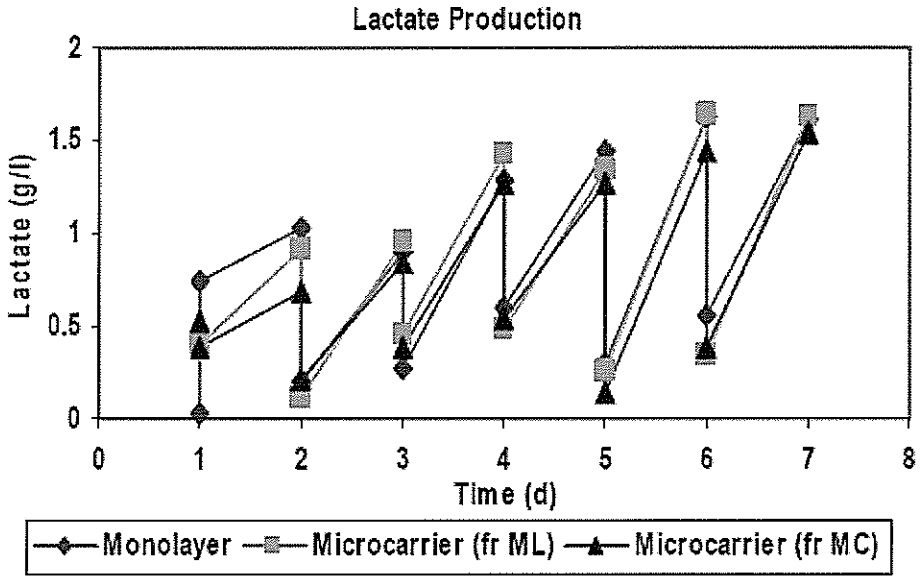

Glutamine Consumption
Glutamine (mM)
1
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
7
8
Time (d)

FIGURE 16 (CONTINUED)



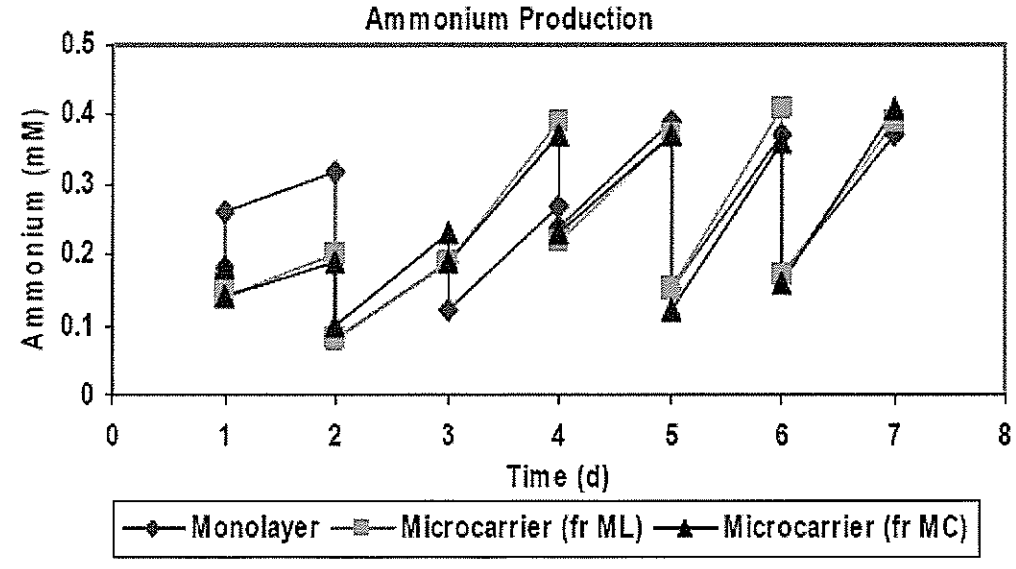
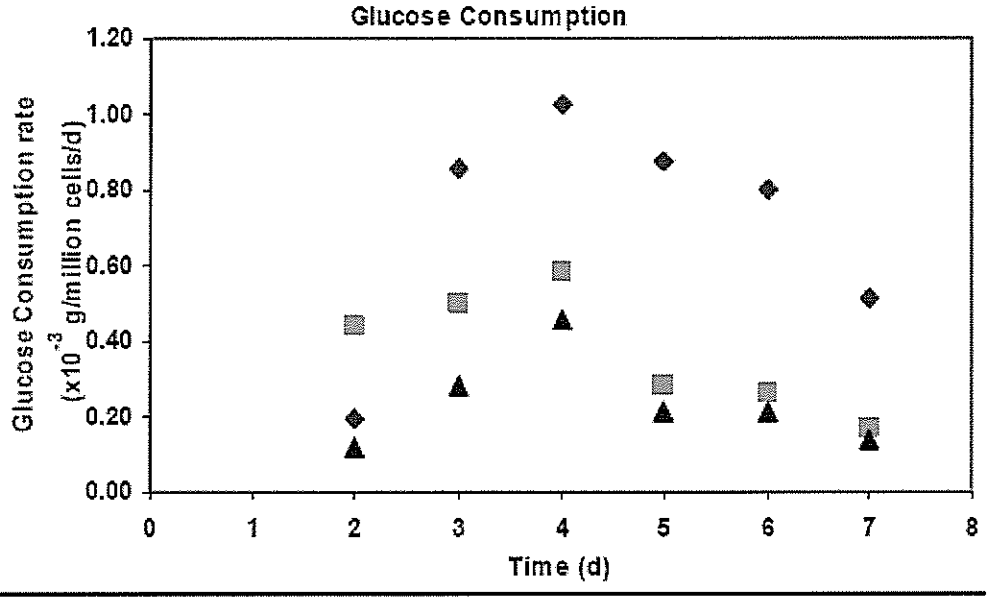


FIGURE 17


Glucose Consumption
Glucose Consumption rate (x10-3 g/million cells/d)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0
1
2
3
4
5
6
7
8
Time (d)

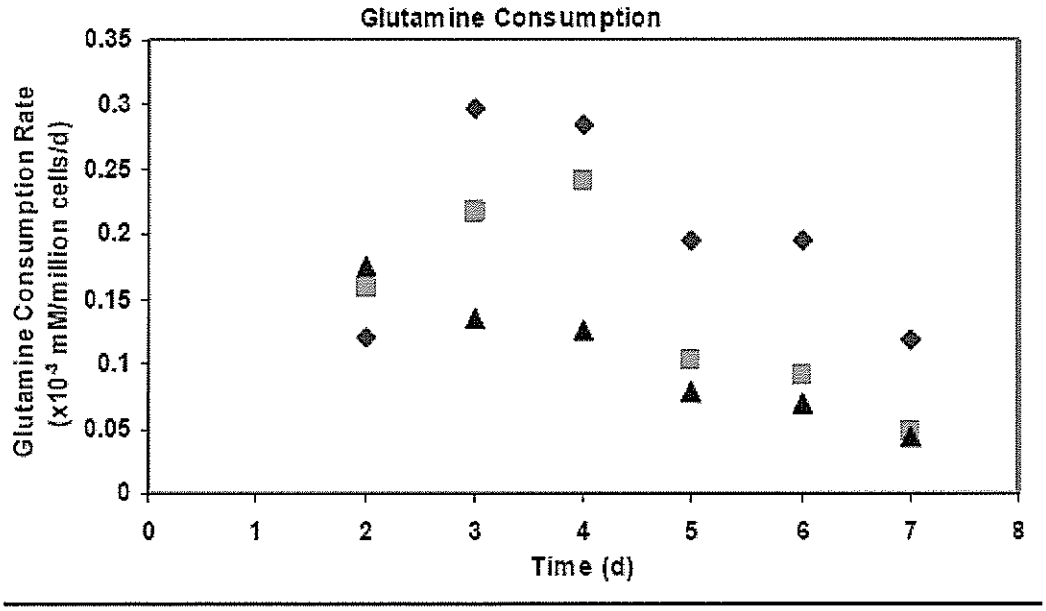
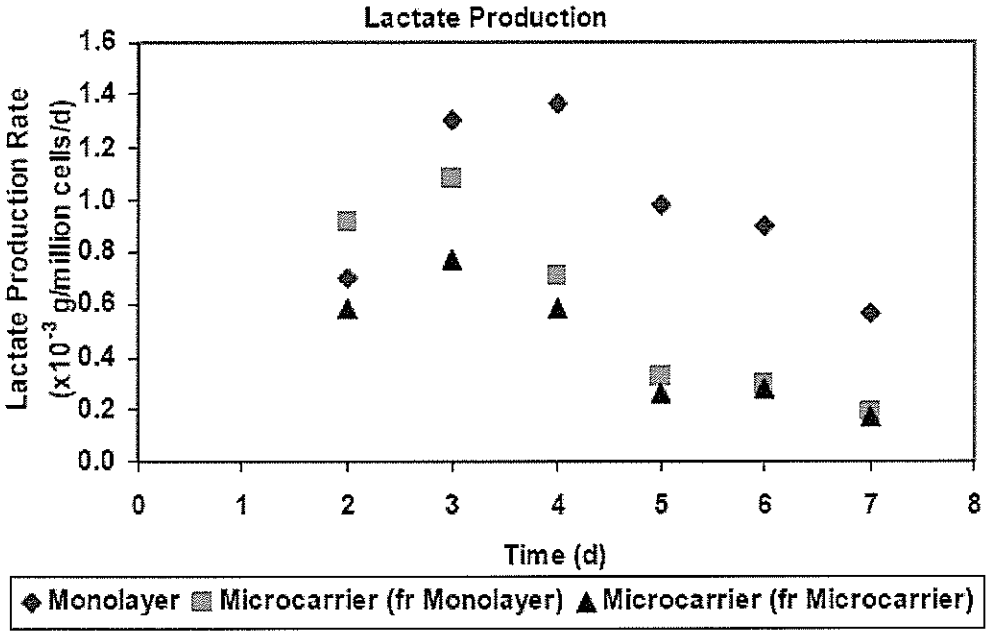

Glutamine Consumption
Glutamine Consumption Rate (x10-3 mM/million cells/d)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
1
2
3
4
5
6
7
8
Time (d)

FIGURE 17 (CONTINUED)



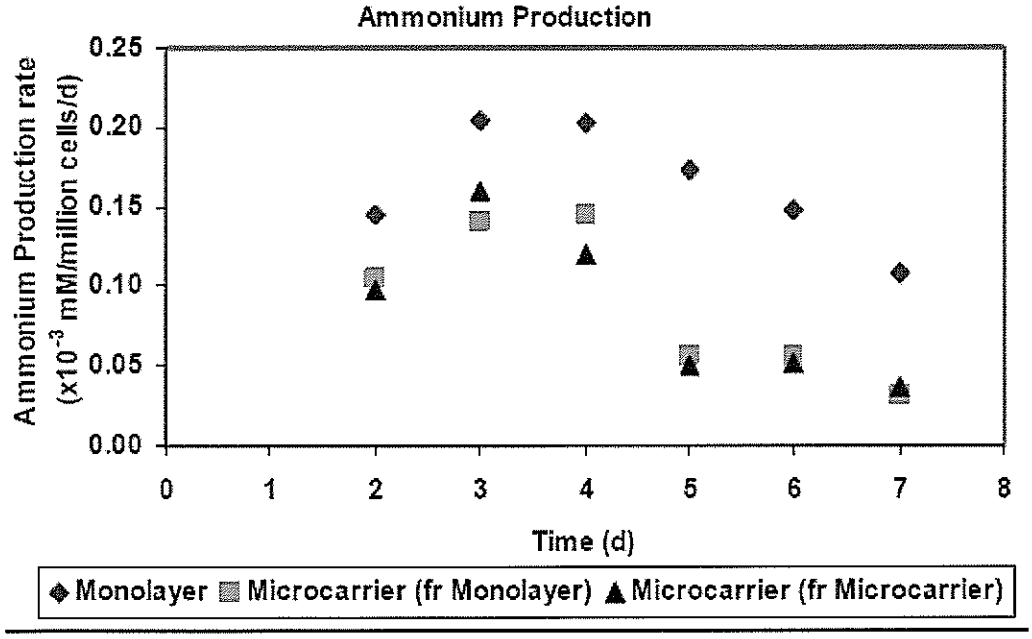


FIGURE 18

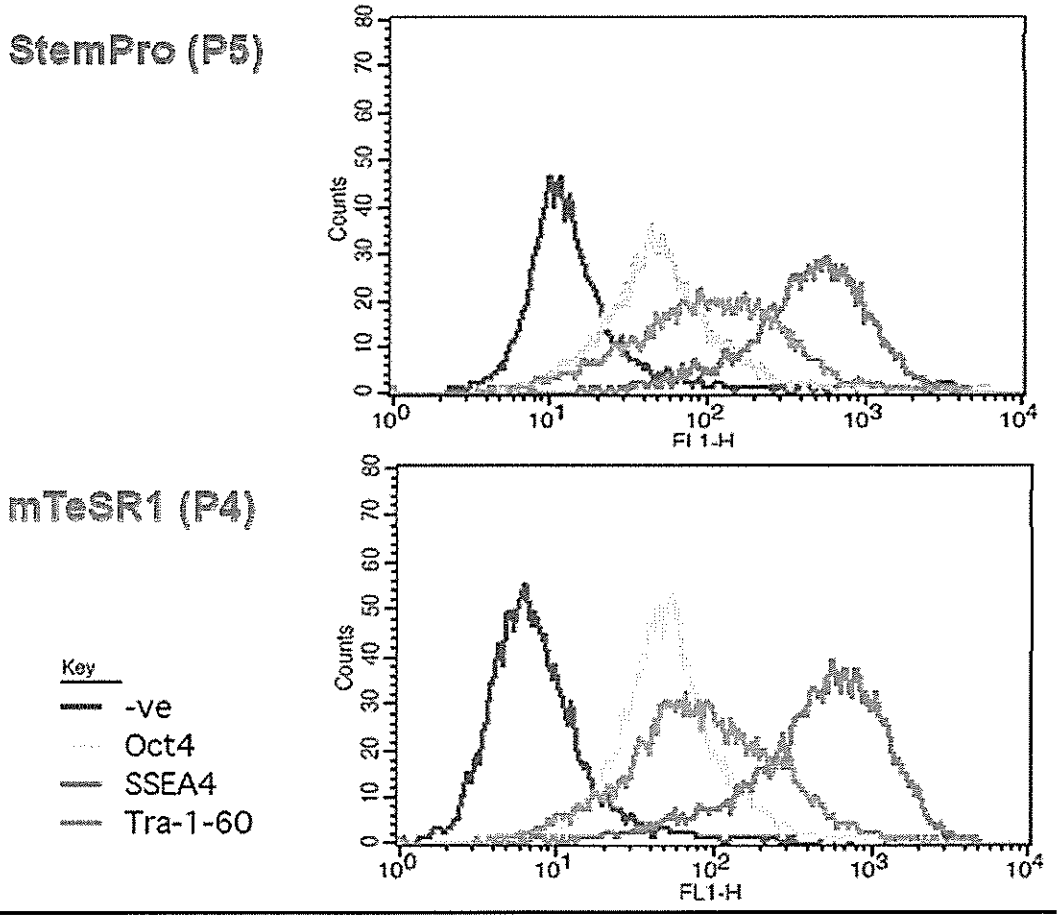


FIGURE 19

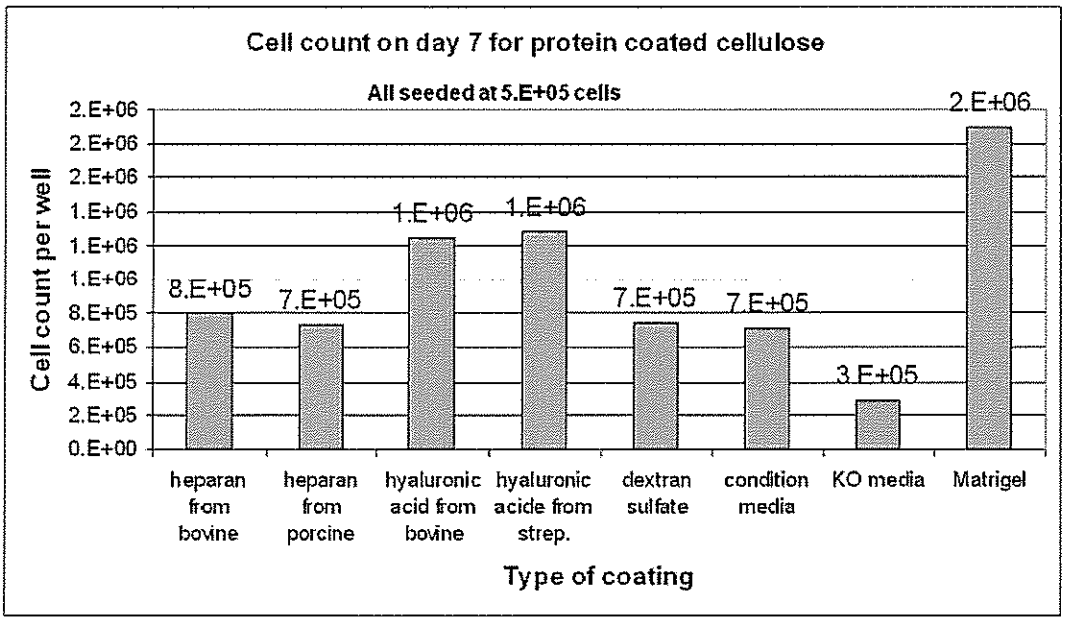

Cell count on day 7 for protein coated cellulose
All seeded at 5.E+05 cells
Cell count per well
2.E+06
2.E+06
2.E+06
1.E+06
1.E+06
1.E+06
8.E+05
6.E+05
4.E+05
2.E+05
0.E+00
8.E+05
7.E+05
1.E+06
1.E+06
7.E+05
7.E+05
3.E+05
2.E+06
heparan from bovine
heparan from porcine
hyaluronic acid from bovine
hyaluronic acide from strep.
dextran sulfate
condition media
KO media
Matrigel
Type of coating

FIGURE 20

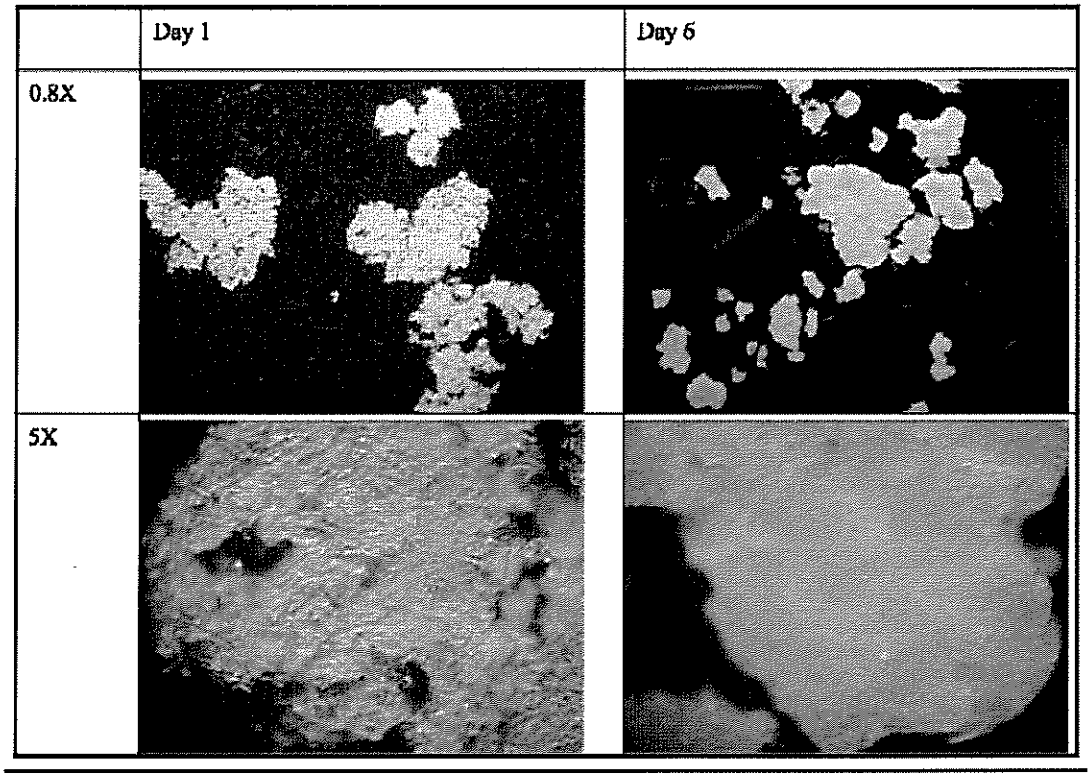
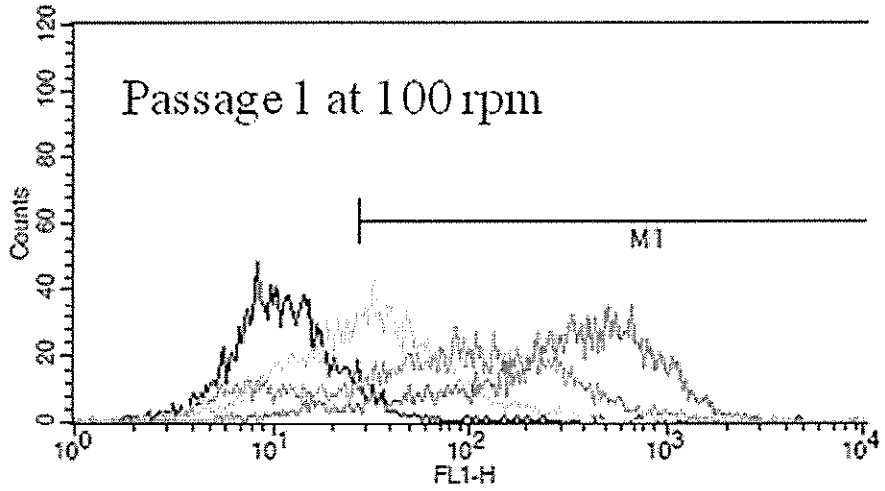
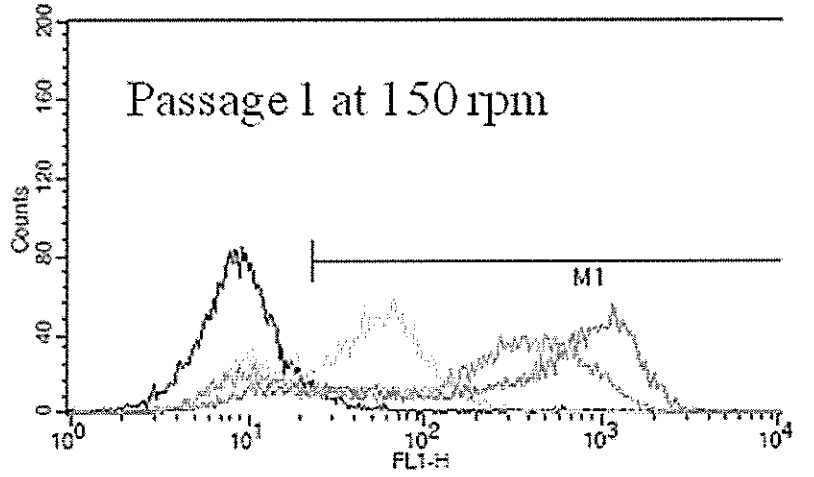


FIGURE 21A



Key	Name	Parameter
——	CONTROL	
. . .	OCT 4 - 56.5%	
___	SSEA 4 - 96%	
___	TRA 1 60 - 75.4%	

FIGURE 21B



Key	Name	Parameter
——	CONTROL	68%
	OCT-4	88%
___	SSEA-4	78%
___	TRA-1-60	

FIGURE 22

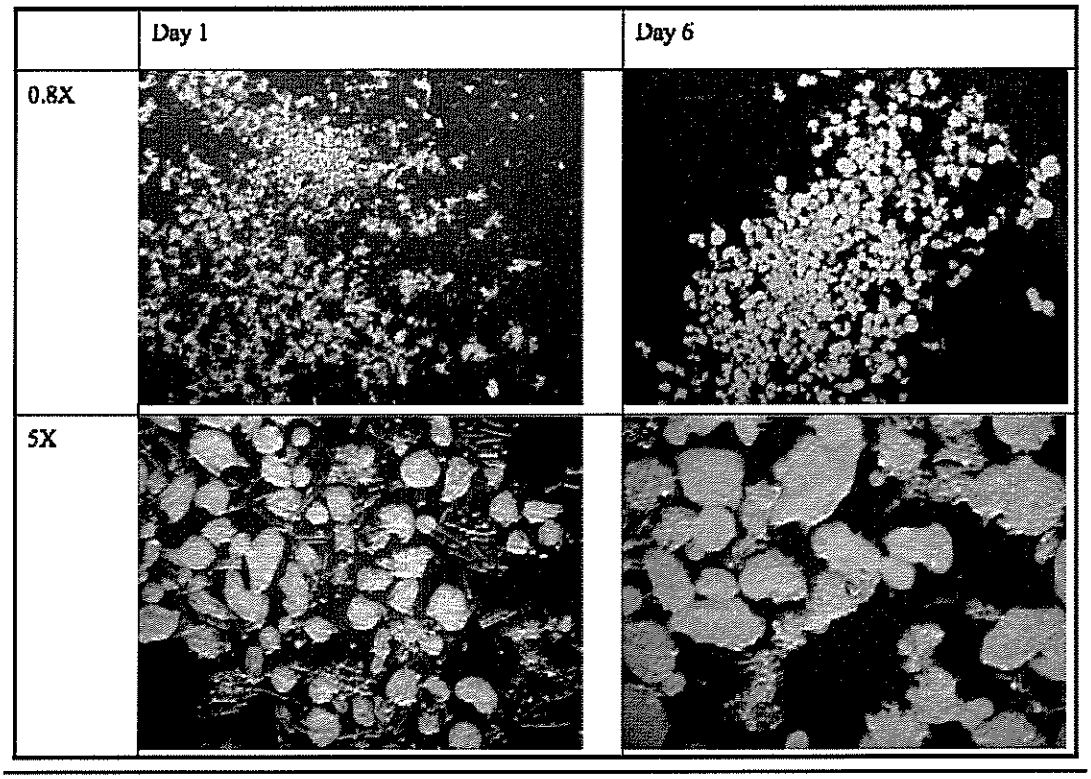
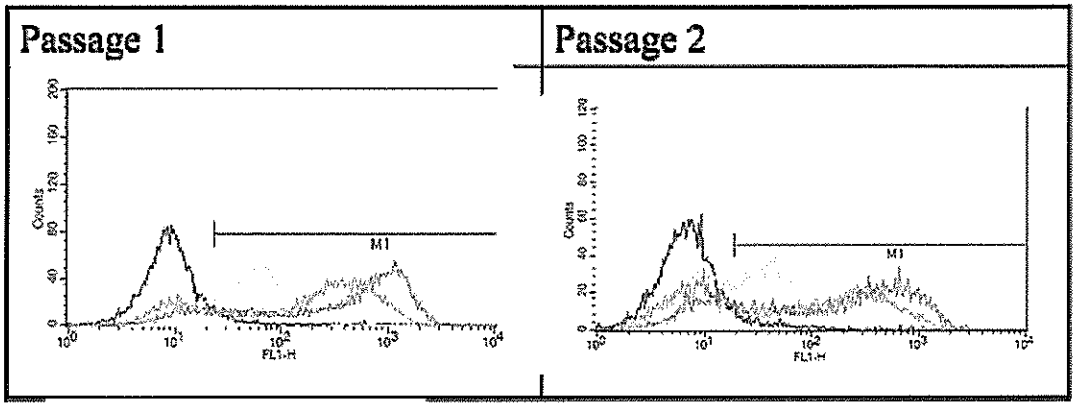


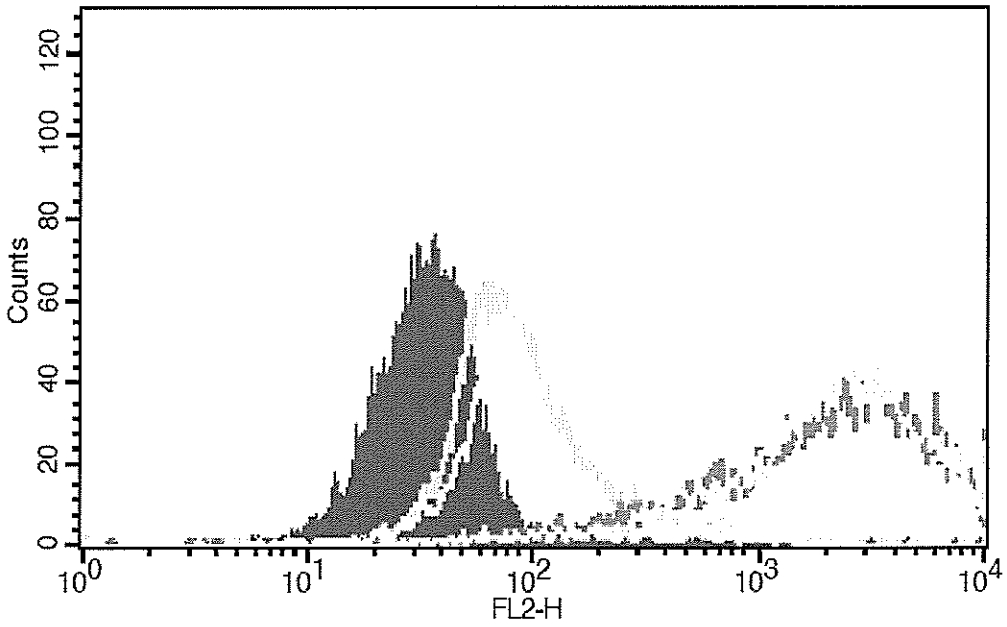
FIGURE 23



Key	Name	Parameter
—	CONTROL	
	OCT-4	68%
—	SSEA-4	88%
—	TRA-1-60	78%

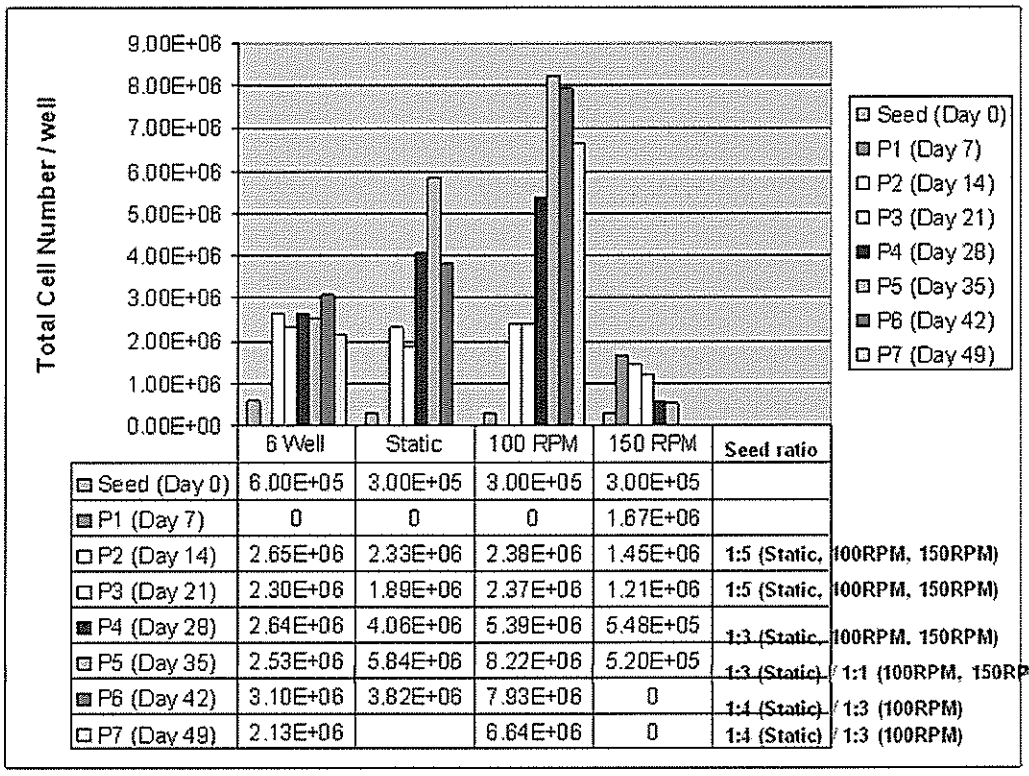
Key	Name	Parameter
—	control	
	Oct 4- 57.5%	
—	SSEA 4- 75.6%	
—	Tra 1 60- 69.8%	

FIGURE 24



Key	Name	
	071212.004	control
	071212.005	Oct3/4 (Static)
	071212.006	Tra 1-60 (Static)
	071212.002	Oct3/4 (150rpm)
	071212.003	Tra 1-60 (150rpm)

FIGURE 25



	6 Well	Static	100 RPM	150 RPM	Seed ratio
Seed (Day 0)	6.00E+05	3.00E+05	3.00E+05	3.00E+05	
P1 (Day 7)	0	0	0	1.67E+06	
P2 (Day 14)	2.65E+06	2.33E+06	2.38E+06	1.45E+06	1:5 (Static, 100RPM, 150RPM)
P3 (Day 21)	2.30E+06	1.89E+06	2.37E+06	1.21E+06	1:5 (Static, 100RPM, 150RPM)
P4 (Day 28)	2.64E+06	4.06E+06	5.39E+06	5.48E+05	1:3 (Static, 100RPM, 150RPM)
P5 (Day 35)	2.53E+06	5.84E+06	8.22E+06	5.20E+05	1:3 (Static) / 1:1 (100RPM, 150RPM)
P6 (Day 42)	3.10E+06	3.82E+06	7.93E+06	0	1:4 (Static) / 1:3 (100RPM)
P7 (Day 49)	2.13E+06		6.64E+06	0	1:4 (Static) / 1:3 (100RPM)

FIGURE 26A

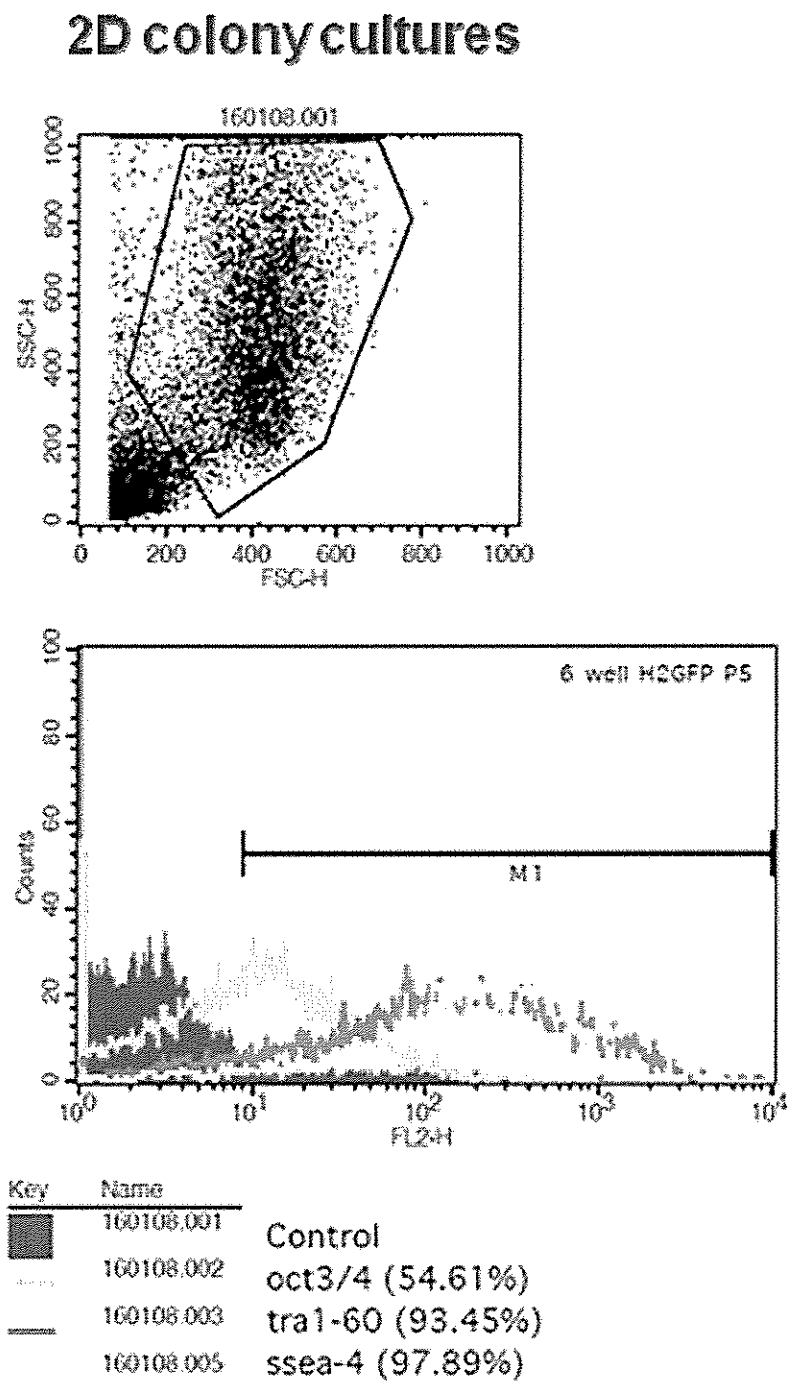

2D colony cultures
160108.001
SSC-H
FSC-H
0
200
400
600
800
1000
6 well H2GFP P5
M1
Counts
FL2-H
Key
Name
160108.001
160108.002
160108.003
160108.005
Control
oct3/4 (54.61%)
tra1-60 (93.45%)
ssea-4 (97.89%)

FIGURE 26B

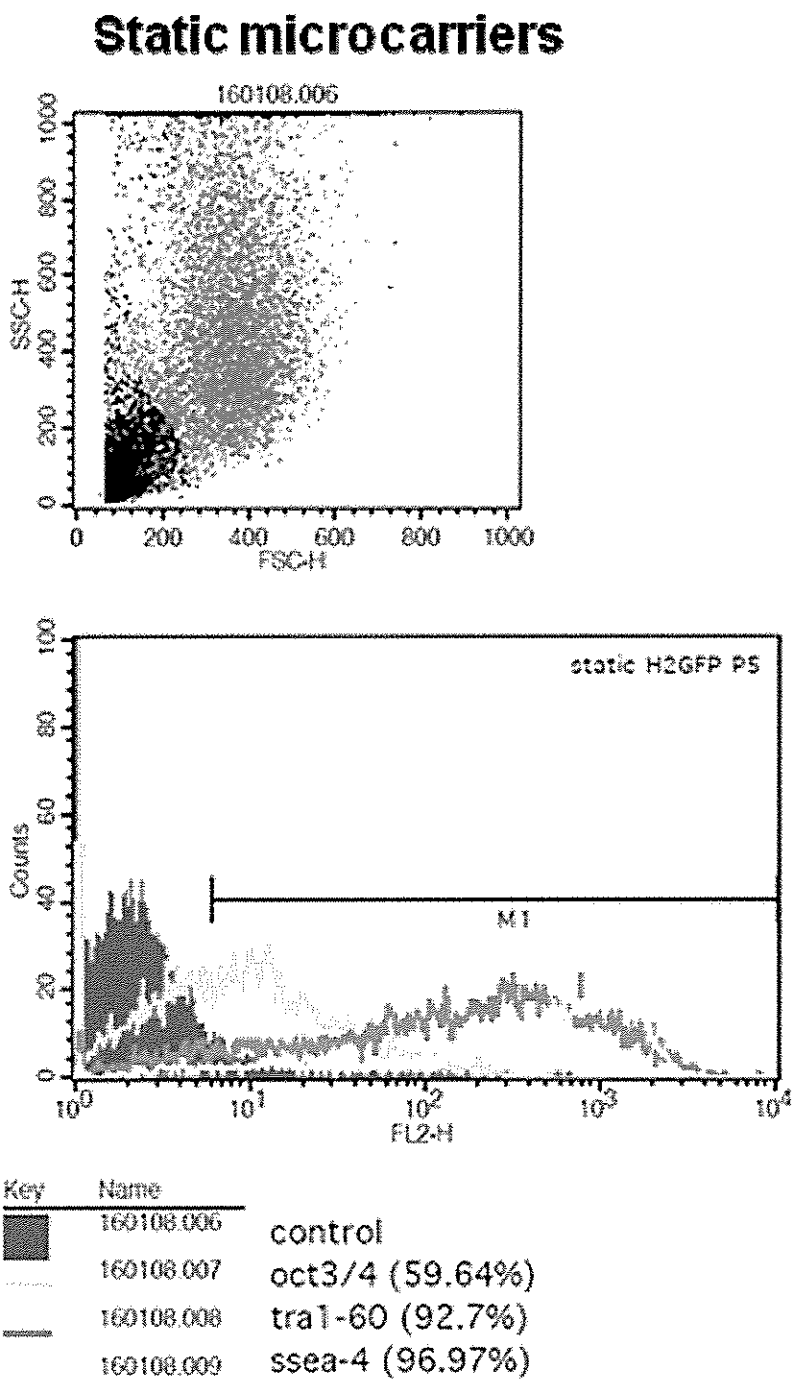

Static microcarriers
160108.006
SSC-H
FSC-H
0
200
400
600
800
1000
static H2GFP P5
Counts
M1
FL2-H
Key
Name
160108.006
160108.007
160108.008
160108.009
control
oct3/4 (59.64%)
tra1-60 (92.7%)
ssea-4 (96.97%)

FIGURE 26C

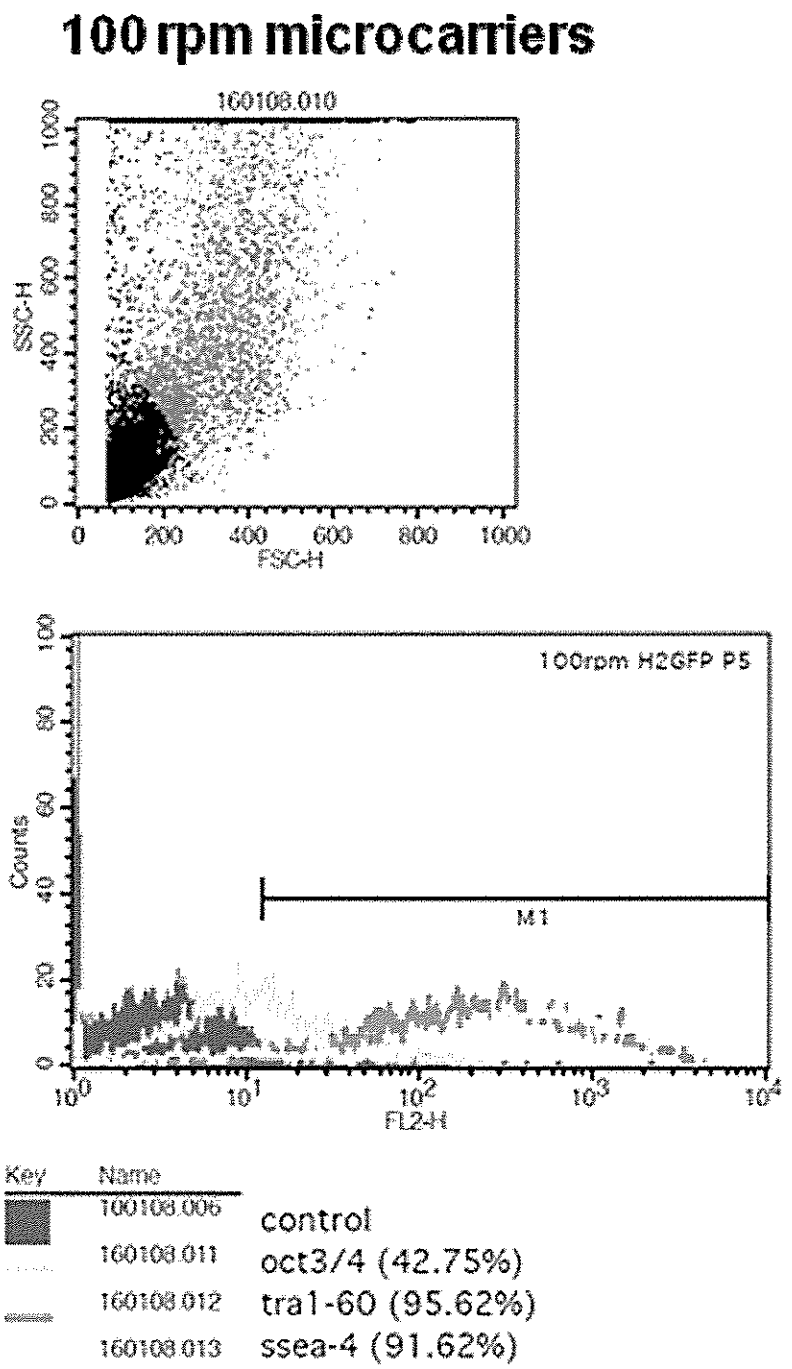
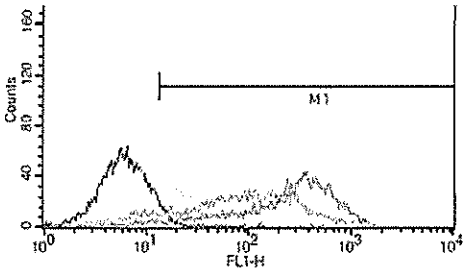

100 rpm microcarriers
160108.010
SSC-H
FSC-H
100rpm H2GFP P5
Counts
M1
FL2-H
Key
Name
100108.006
control
160108.011
oct3/4 (42.75%)
160108.012
tra1-60 (95.62%)
160108.013
ssea-4 (91.62%)

FIGURE 27A

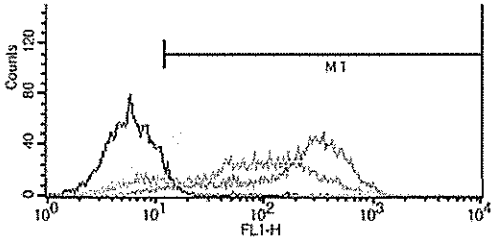
DE52



Key	Name	Parameter	Gate
	Data.009	control	
	Data.010	oct 4 - 42.06%	
	Data.011	ssea 4 - 98.0%	
	Data.012	tra 1 60 - 89.22%	

FIGURE 27B

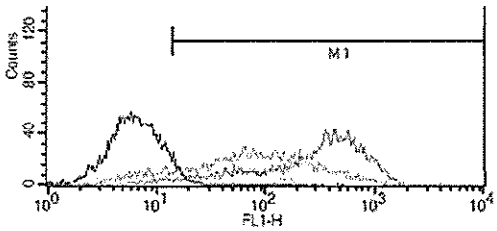
DE53



Key	Name	Parameter	Gate
	Data.013	control	
	Data.014	oct 4 - 62.3%	
	Data.015	ssea 4 - 97.5%	
	Data.016	tra 1 60 - 86.1%	

FIGURE 27C

QA52



Key	Name	Parameter	Gate
	Data.001	control	
	Data.002	oct 4 - 68.6%	
	Data.003	ssea 4 - 97.8%	
	Data.004	tra 1 60 - 88.3%	

FIGURE 28

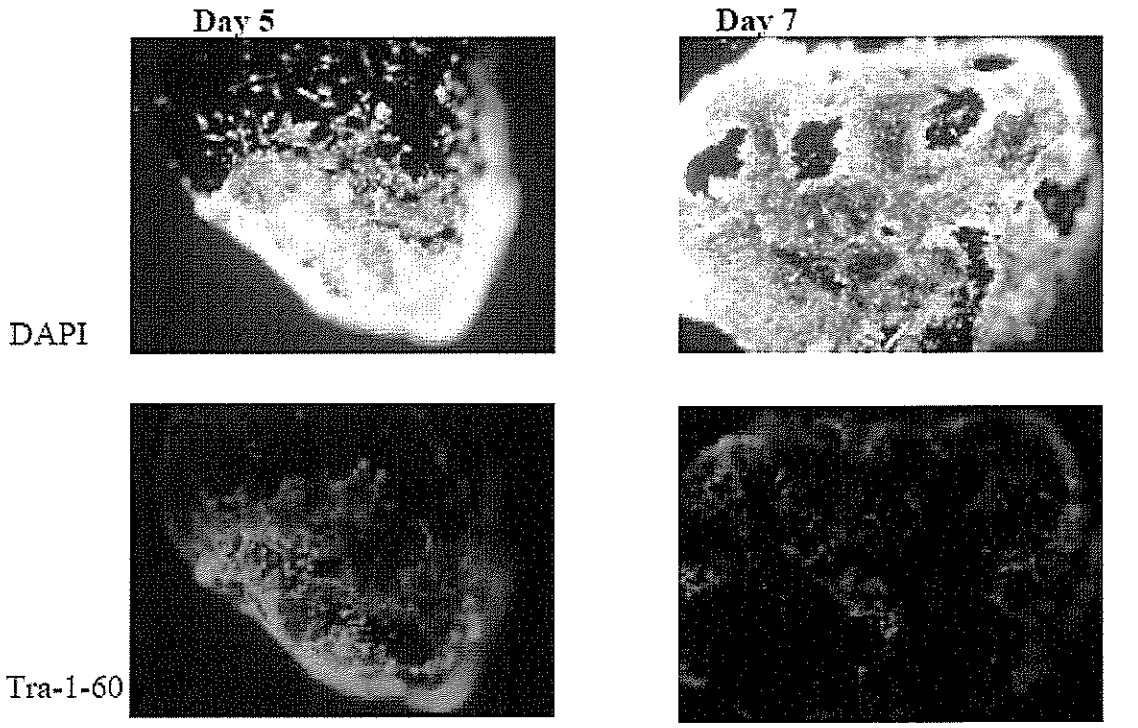


FIGURE 29

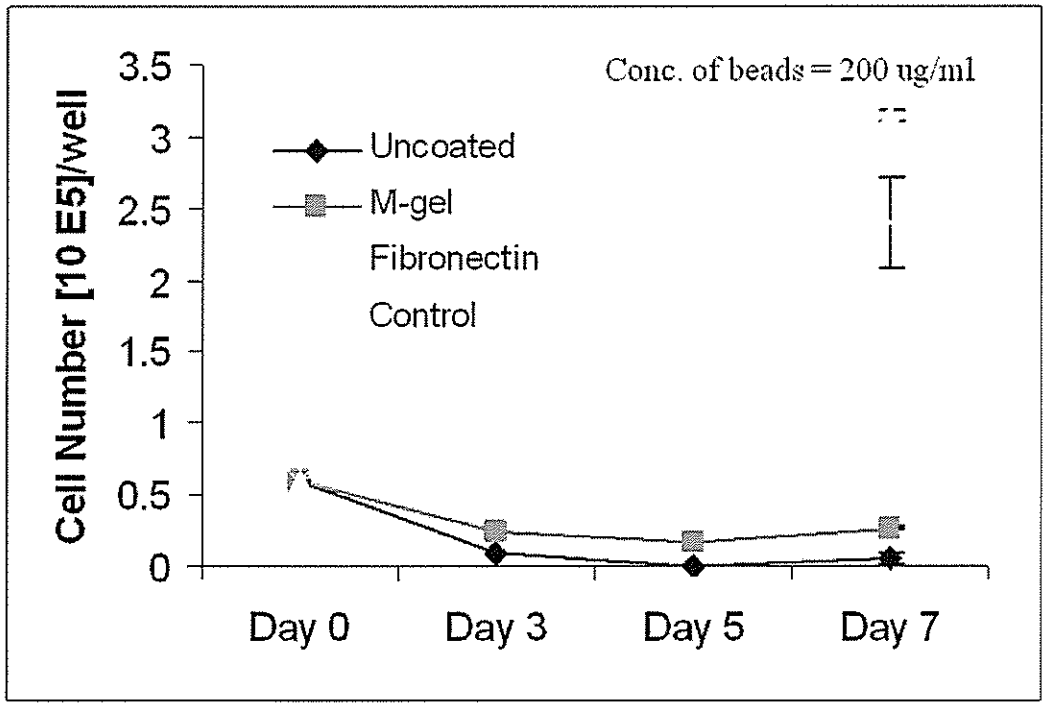

Conc. of beads = 200 ug/ml
Uncoated
M-gel
Fibronectin
Control
Cell Number [10 E5]/well
3.5
3
2.5
2
1.5
1
0.5
0
Day 0
Day 3
Day 5
Day 7

FIGURE 30

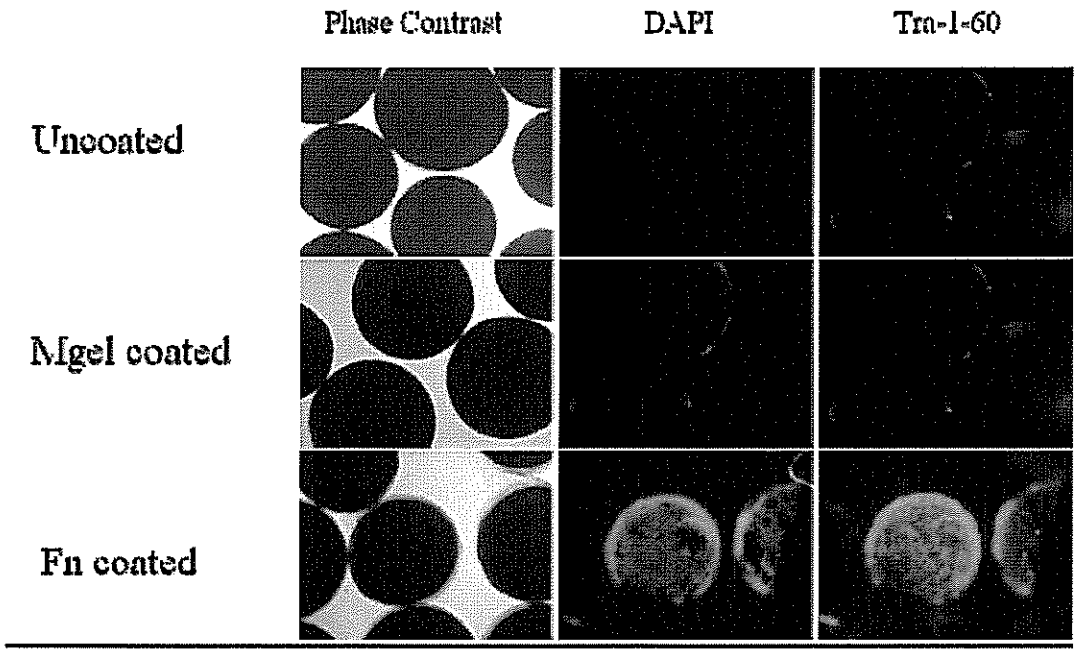


FIGURE 31

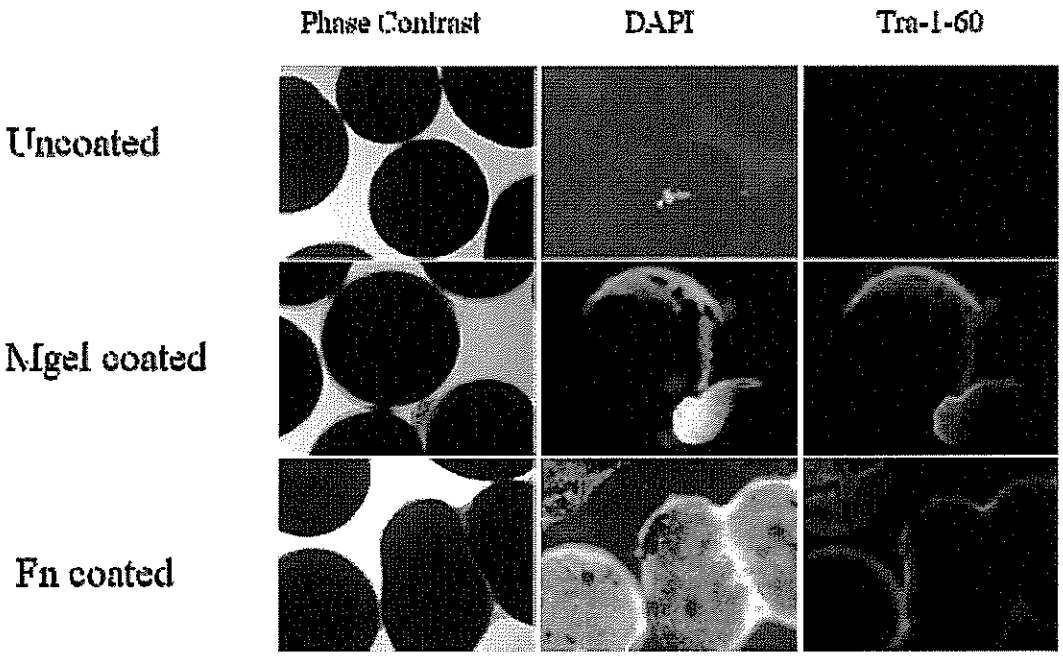


FIGURE 32

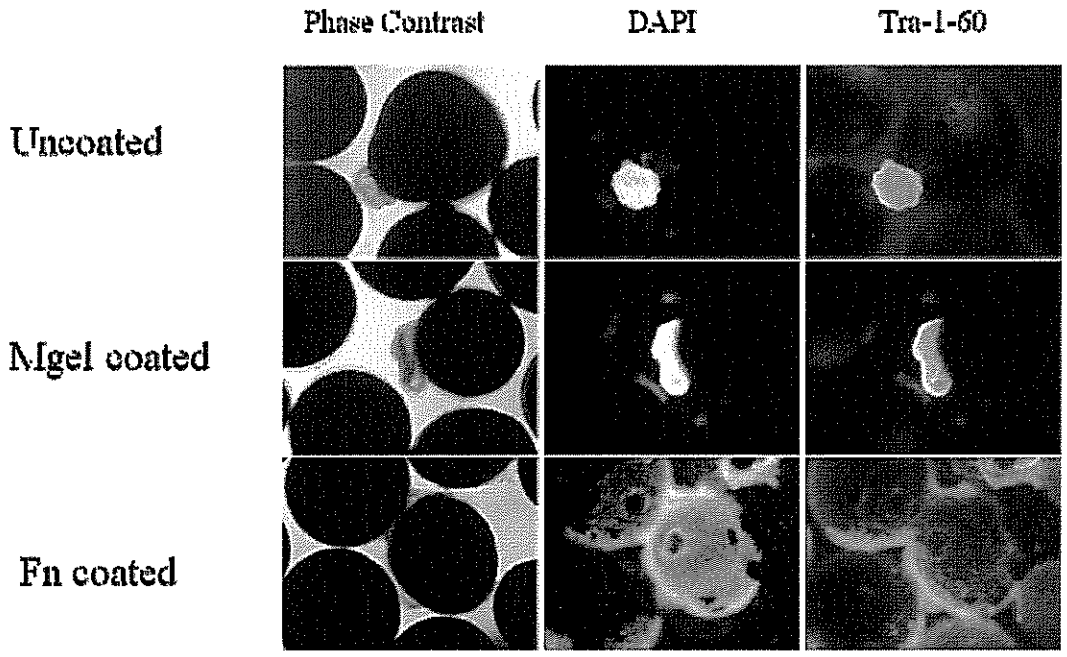


FIGURE 33

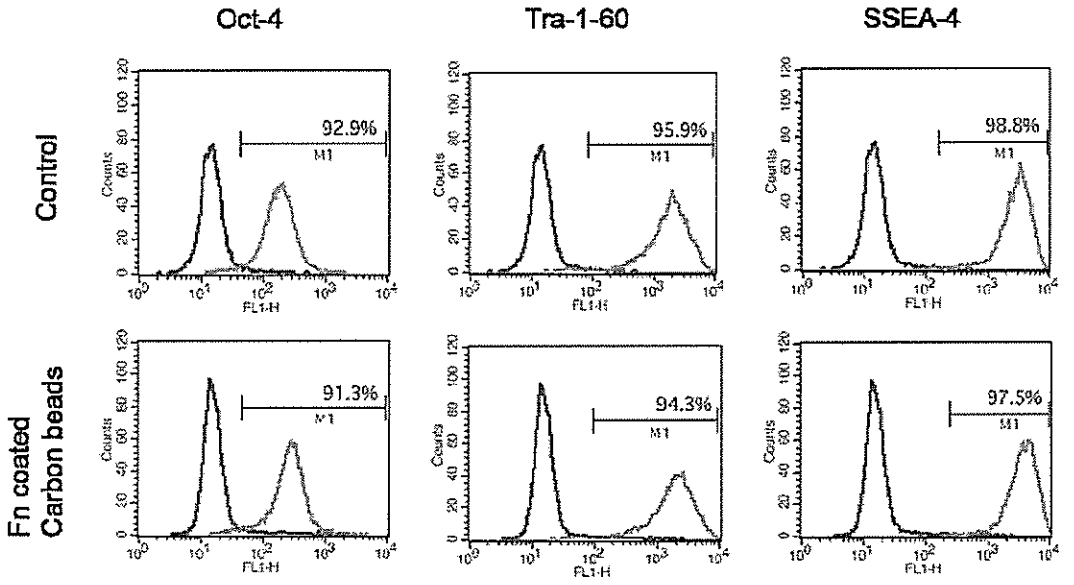

Oct-4
Tra-1-60
SSEA-4
Control
Fn coated Carbon beads
92.9%
95.9%
98.8%
91.3%
94.3%
97.5%
M1
Counts
FL1-H

FIGURE 34A

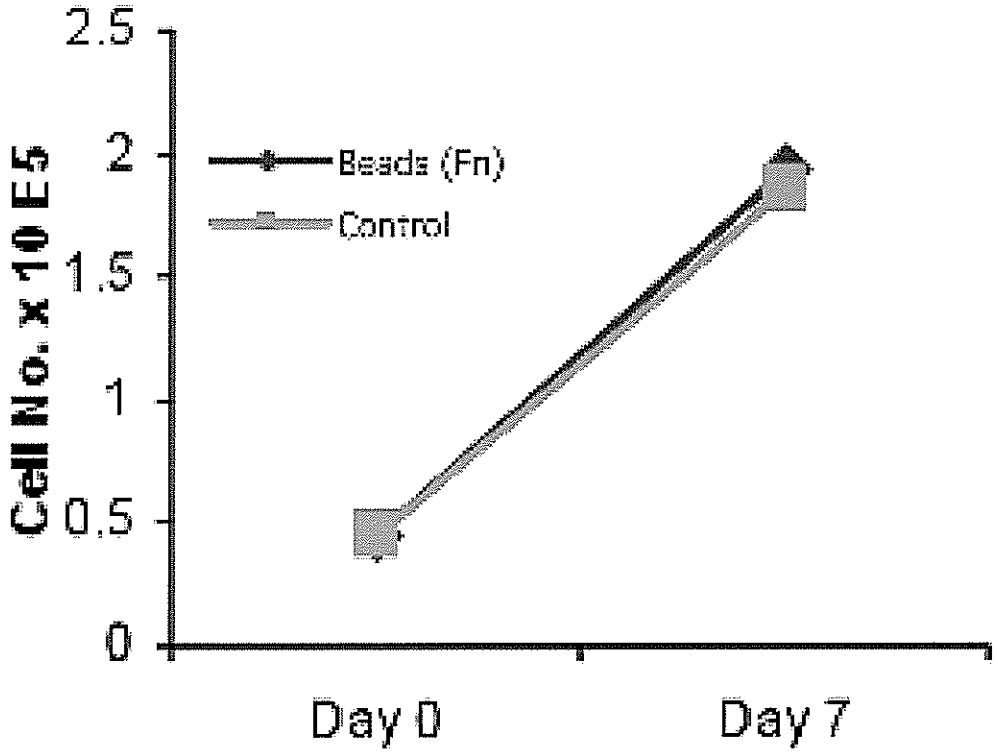
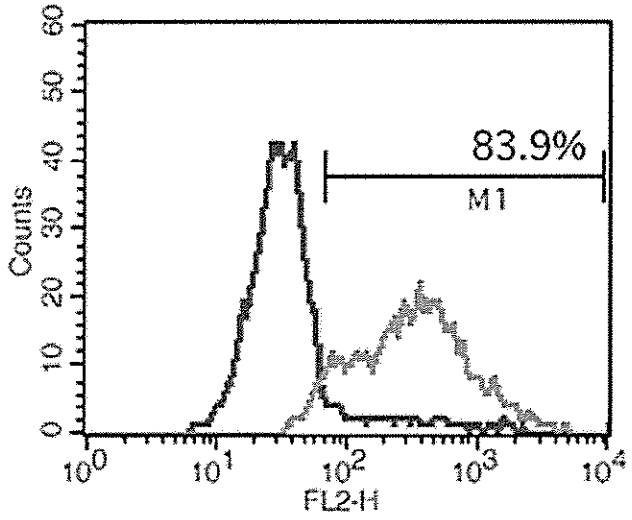
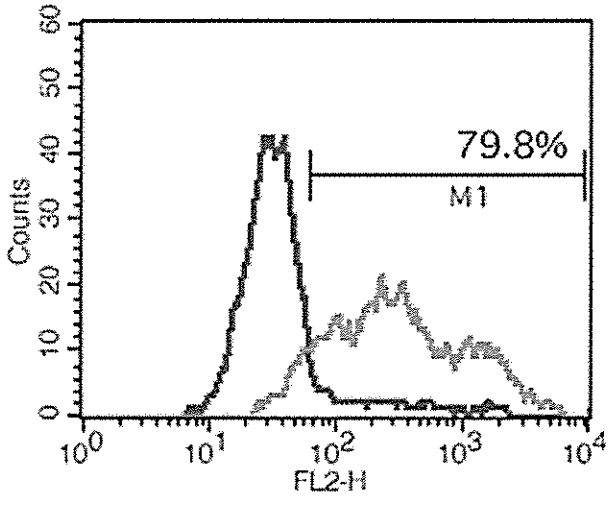


FIGURE 34B

Carbon microcarriers



Control



SUBSTITUTE SHEET (RULE 26)

FIGURE 35

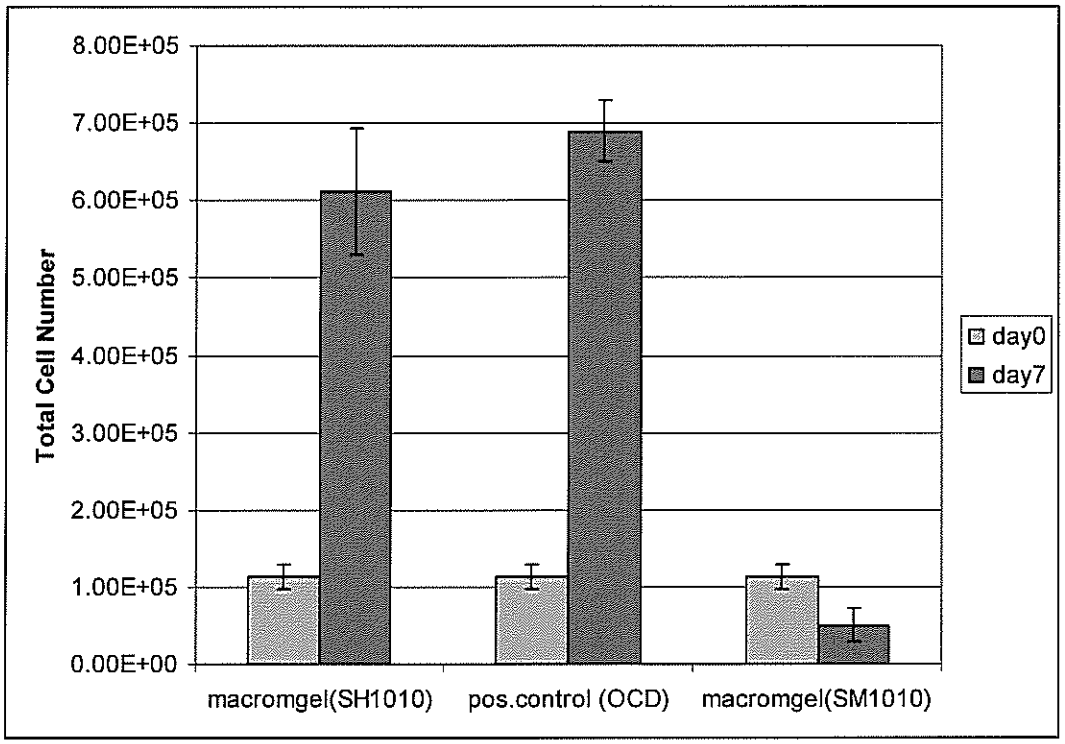

8.00E+05
7.00E+05
6.00E+05
5.00E+05
4.00E+05
3.00E+05
2.00E+05
1.00E+05
0.00E+00
Total Cell Number
day0
day7
macromgel(SH1010)
pos.control (OCD)
macromgel(SM1010)

FIGURE 36

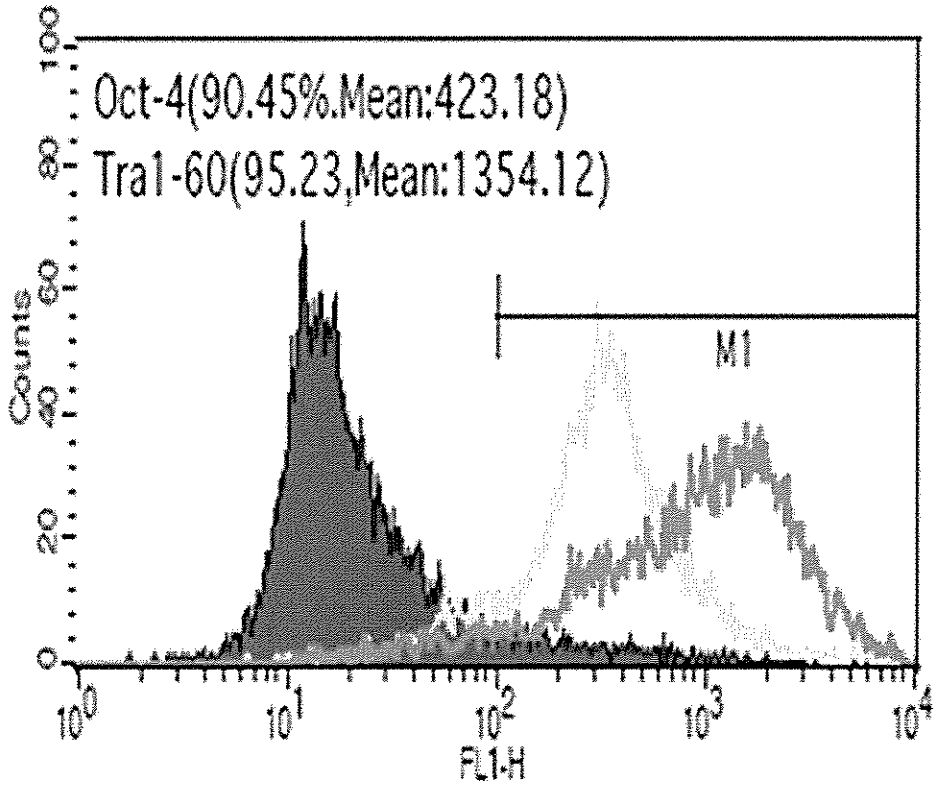
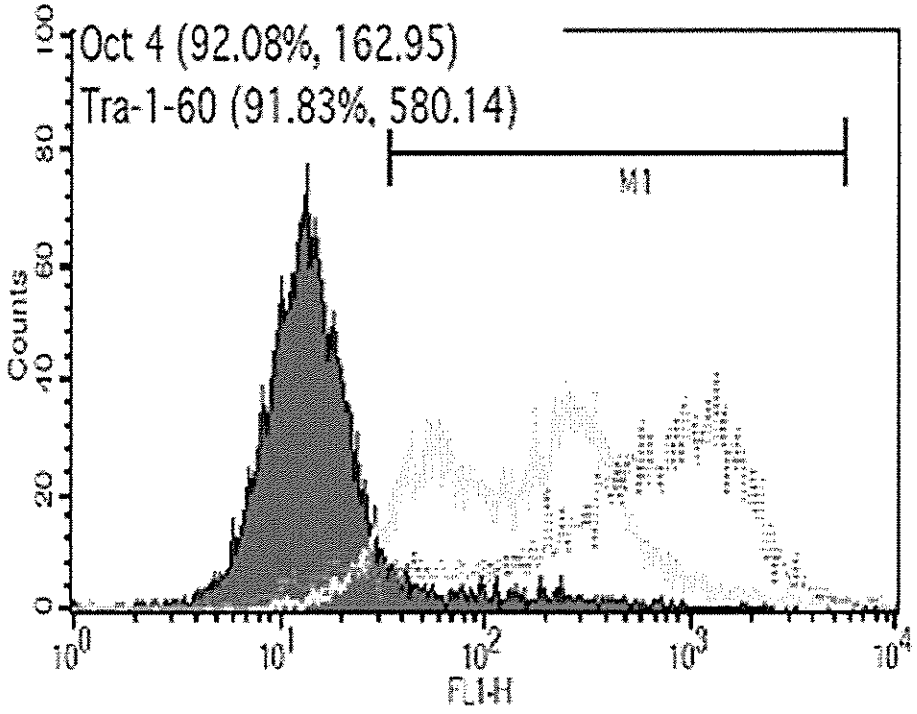

Oct-4(90.45%,Mean:423.18)
Tra1-60(95.23,Mean:1354.12)
M1
Counts
FL1-H
Day 0

FIGURE 36 (CONTINUED)



Key	Name	Parameter	Gate
	neg	FL1-H	G2
	Oct 4	FL1-H	G2
	Tra 1-60	FL1-H	G2

FIGURE 37

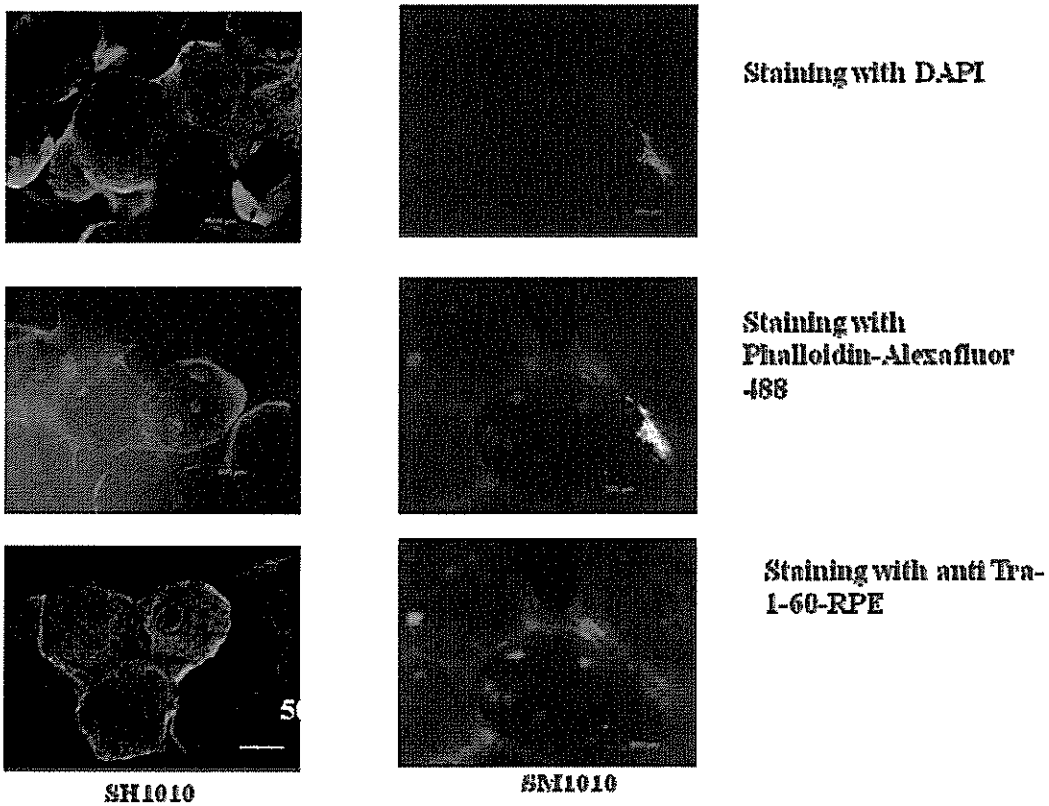

Staining with DAPI
Staining with Phalloidin-Alexafluor 488
Staining with anti Tra-1-60-RPE
SH1010
SH1010

FIGURE 38

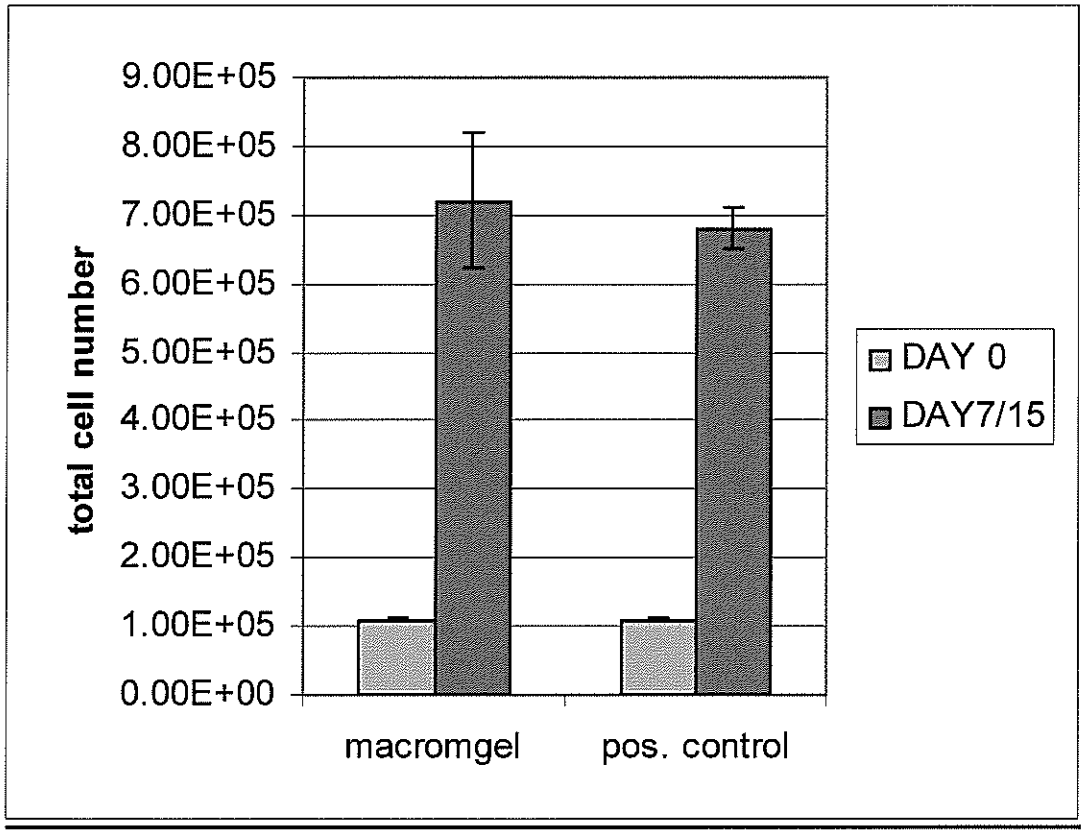
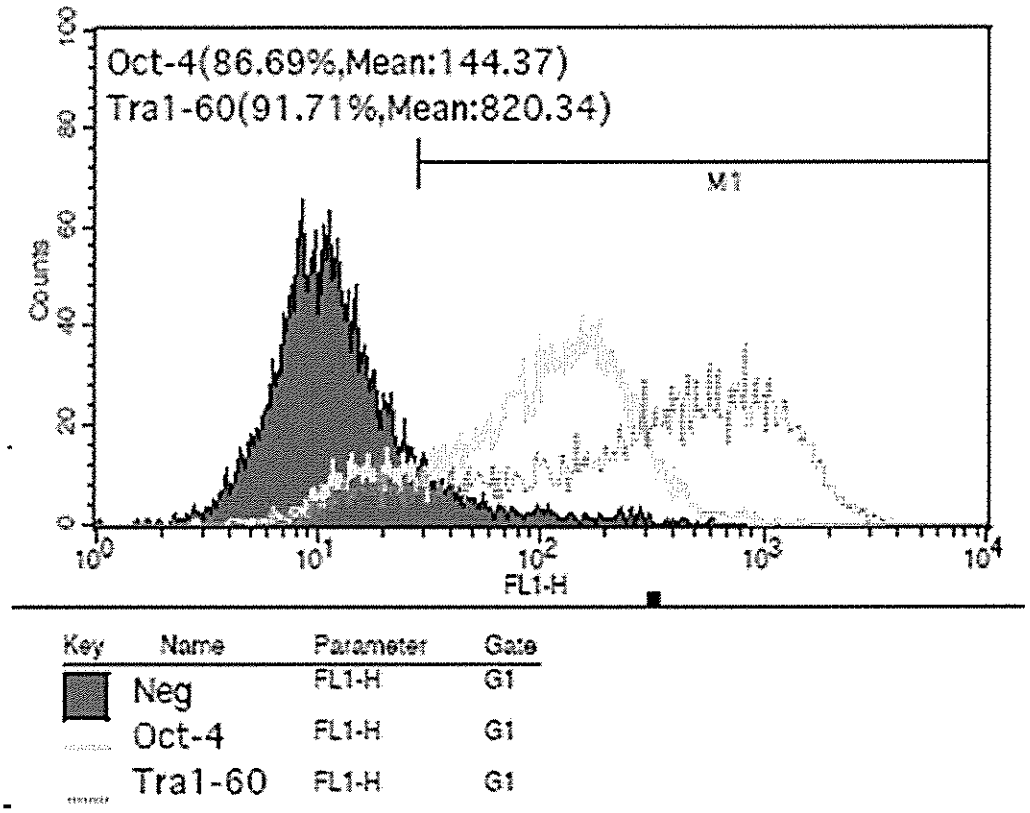
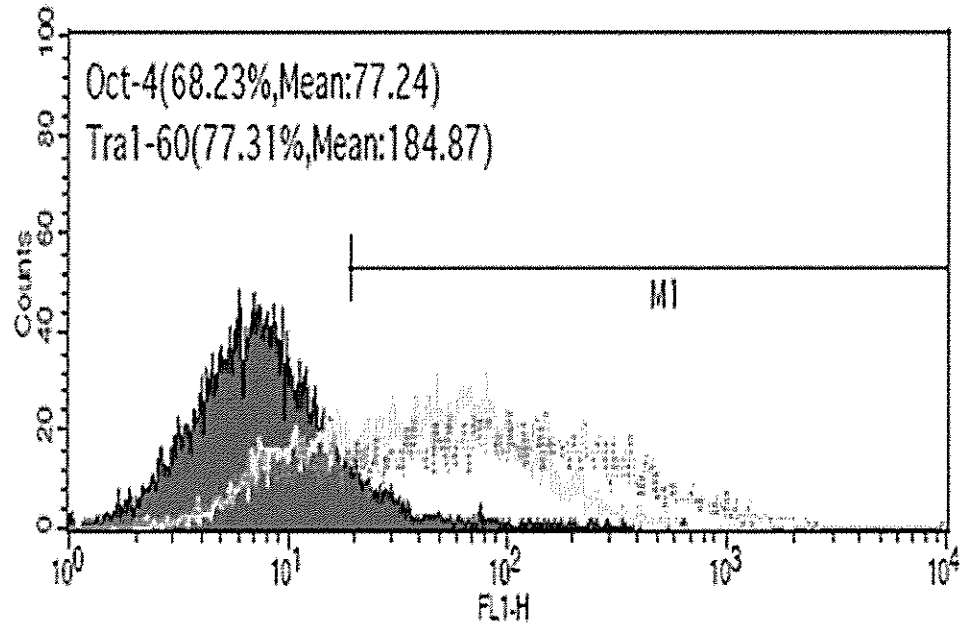

9.00E+05
8.00E+05
7.00E+05
6.00E+05
5.00E+05
4.00E+05
3.00E+05
2.00E+05
1.00E+05
0.00E+00
total cell number
macromgel
pos. control
DAY 0
DAY7/15

FIGURE 39



Day 0

FIGURE 39 (CONTINUED)



Key	Name	Parameter	Gate
	Neg	FL1-H	G1
	Oct-4	FL1-H	G1
	Tra1-60	FL1-H	G1

Day 15

FIGURE 40

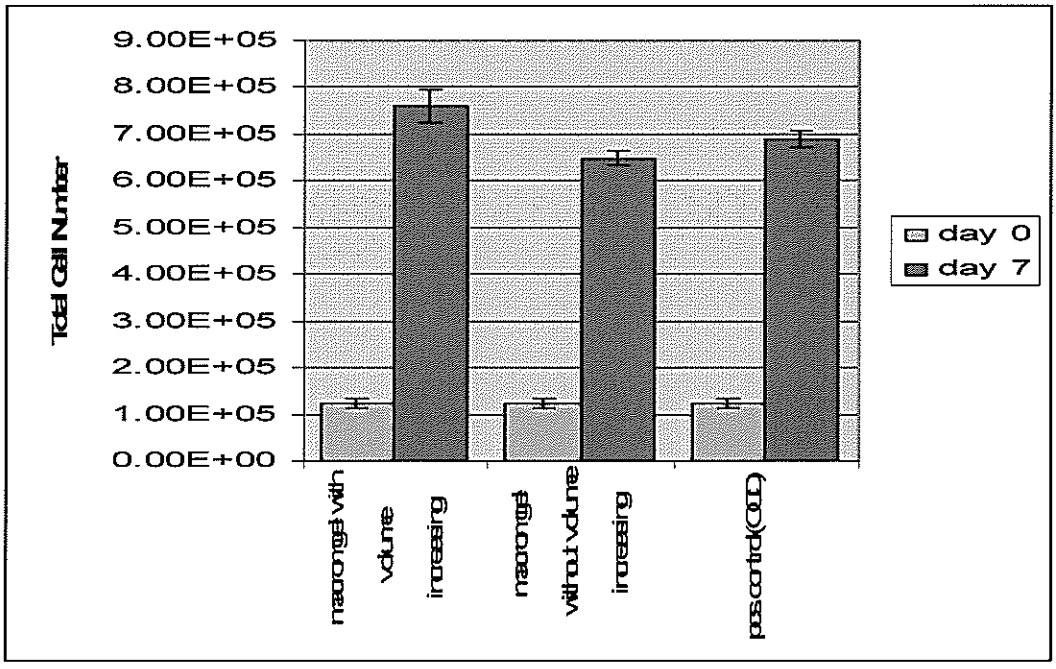

9.00E+05
8.00E+05
7.00E+05
6.00E+05
5.00E+05
4.00E+05
3.00E+05
2.00E+05
1.00E+05
0.00E+00
Total Cell Number
day 0
day 7
macrongs with volume increasing
macrongs without volume increasing
pos control(CCL)

FIGURE 41

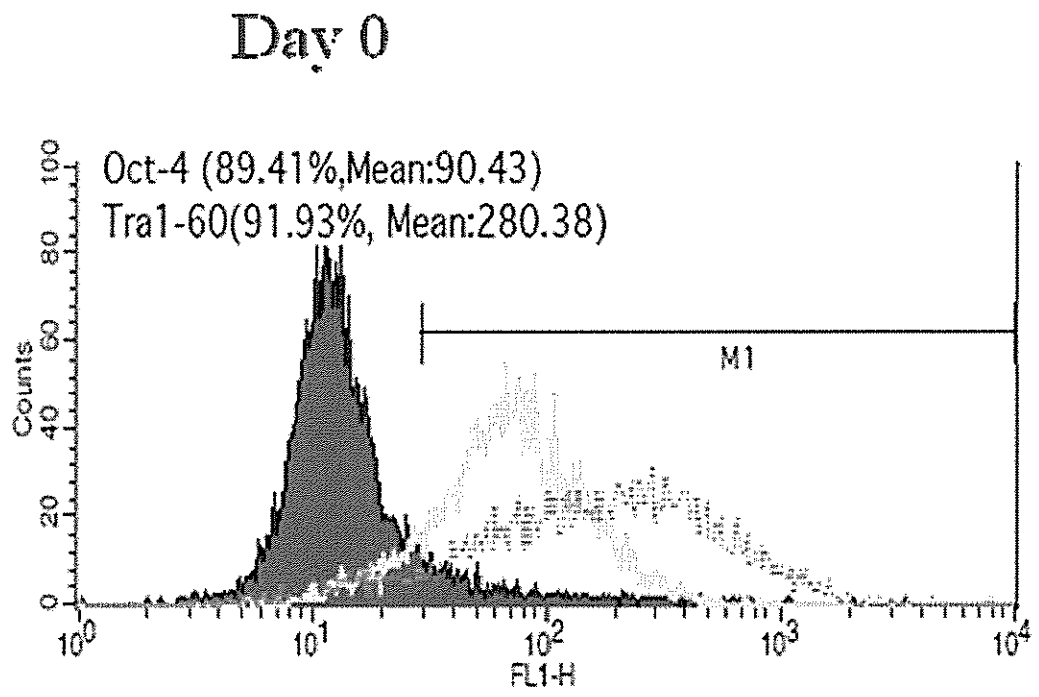

Day 0
Oct-4 (89.41%,Mean:90.43)
Tra1-60(91.93%, Mean:280.38)
M1
Counts
FL1-H
0
20
40
60
80
100
10^0
10^1
10^2
10^3
10^4

FIGURE 41 (CONTINUED)

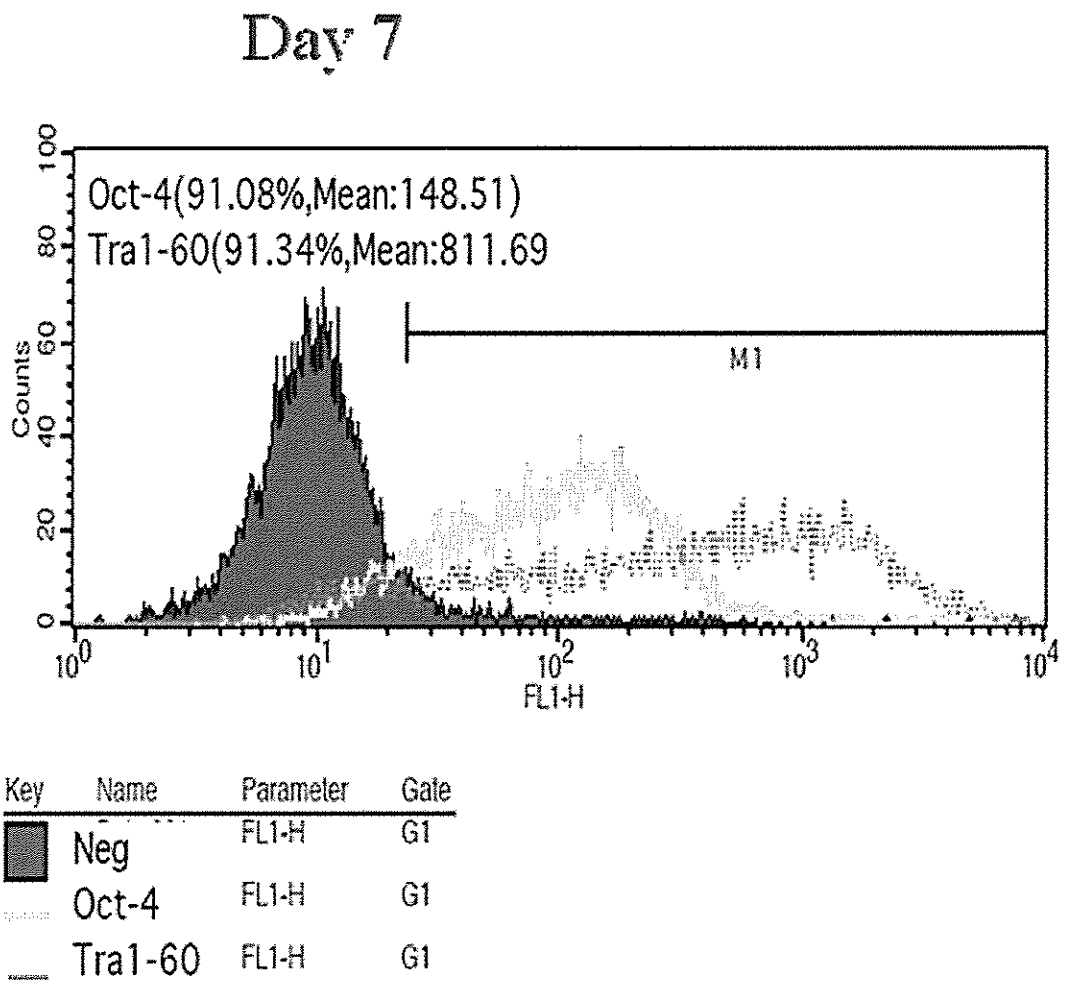
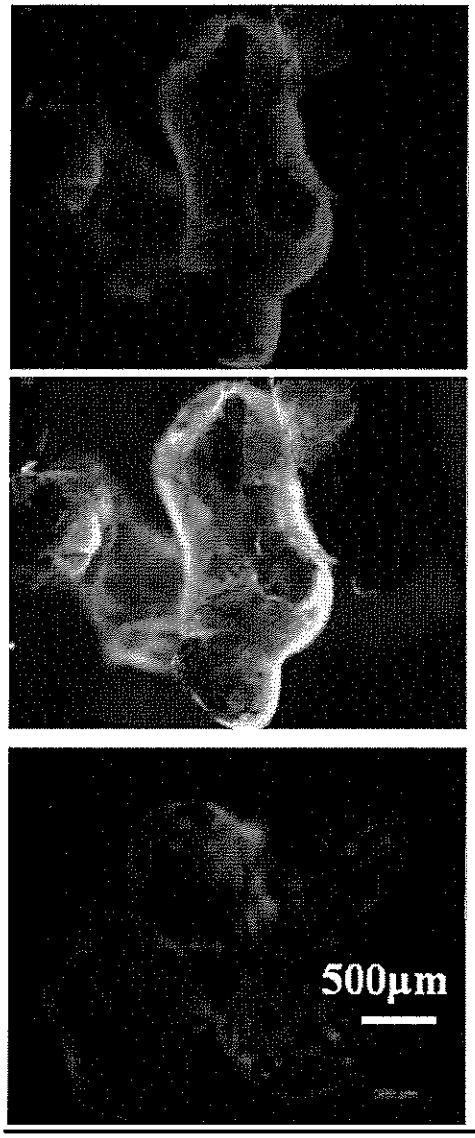


FIGURE 42



Staining with DAPI

Staining with Phalloidin-Alexafluor 488

Staining with anti Tra1-60-RPE

FIGURE 43A

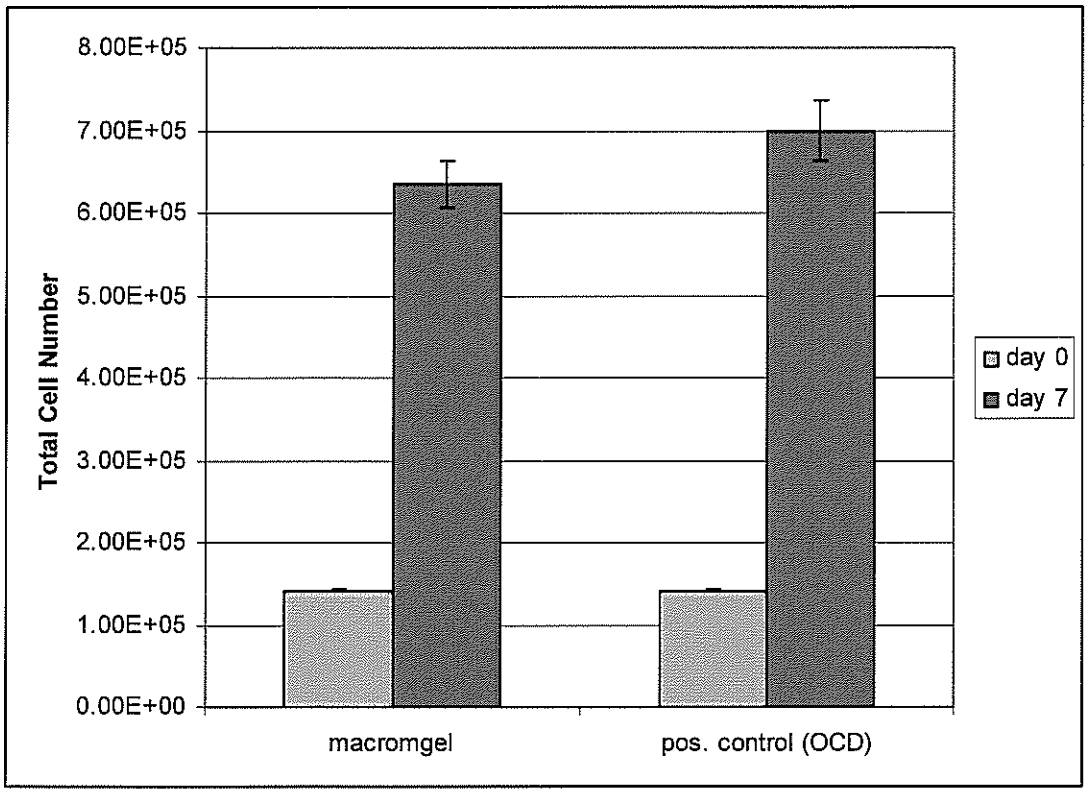

8.00E+05
7.00E+05
6.00E+05
5.00E+05
4.00E+05
3.00E+05
2.00E+05
1.00E+05
0.00E+00
Total Cell Number
macromgel
pos. control (OCD)
day 0
day 7

FIGURE 43B

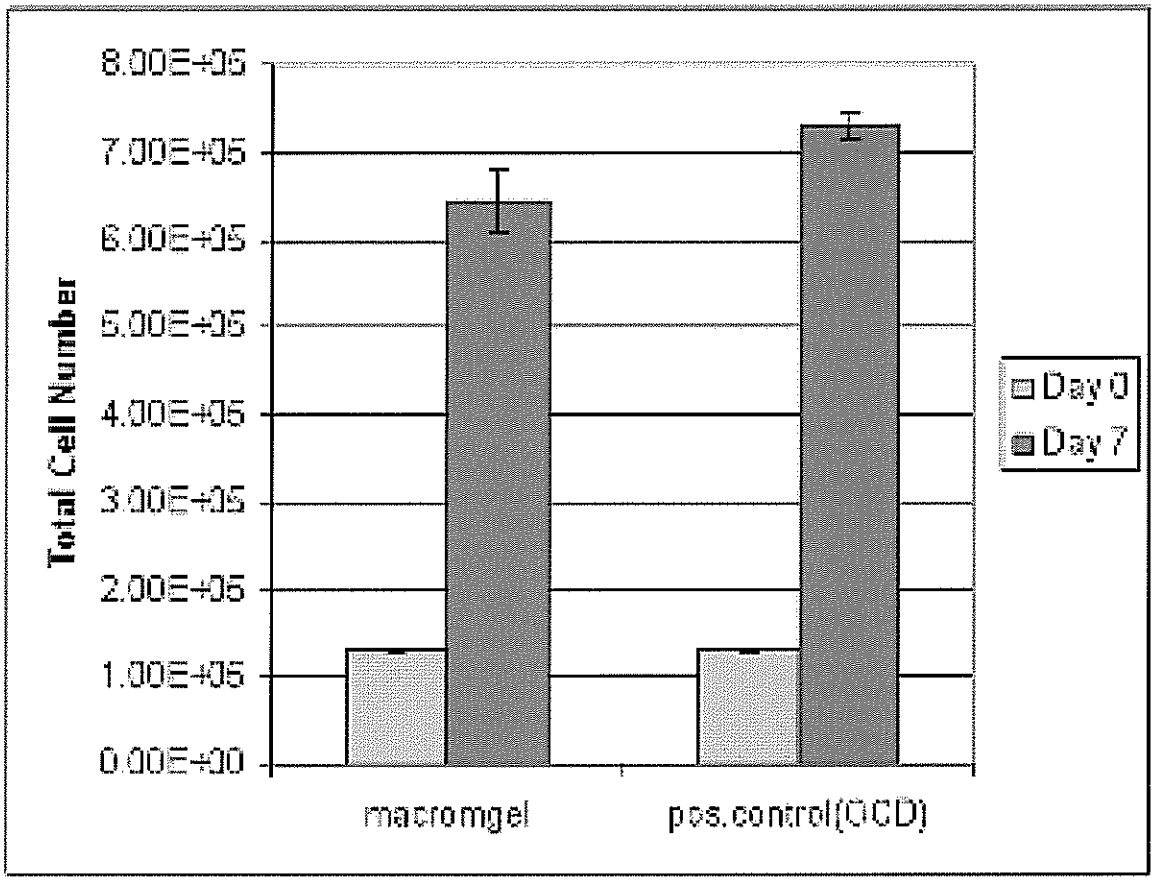
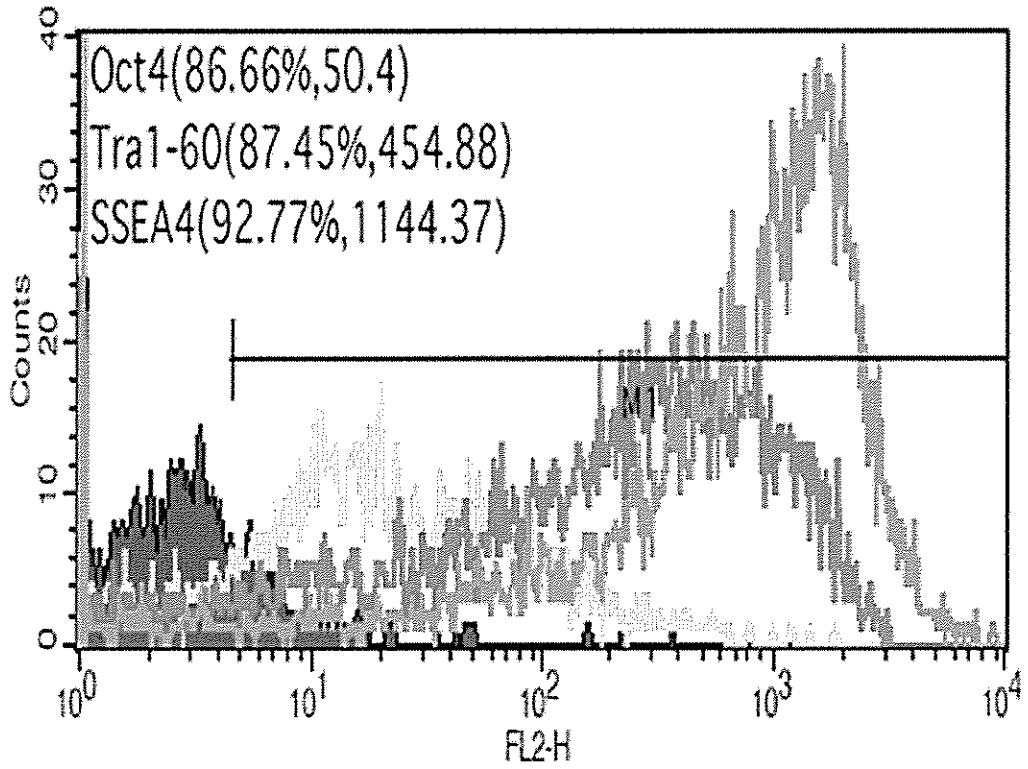
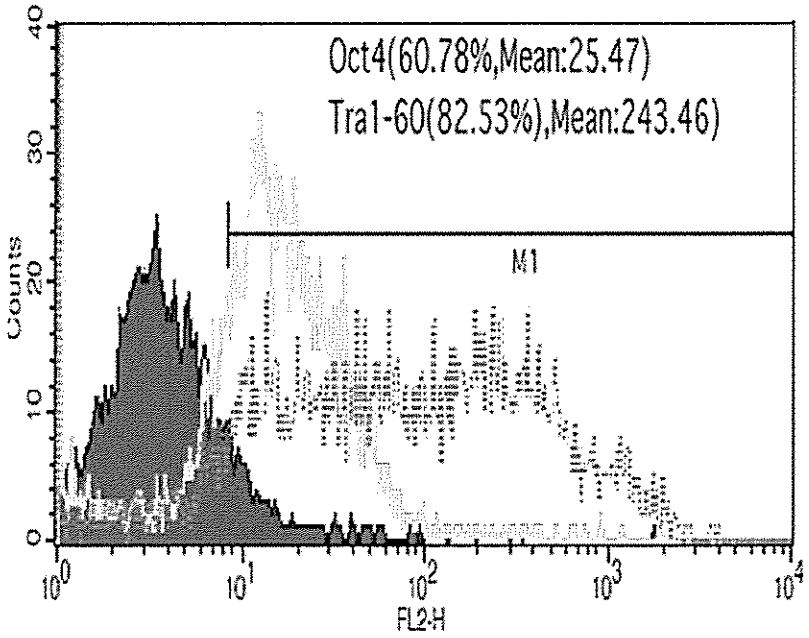

8.00E+05
7.00E+05
6.00E+05
5.00E+05
4.00E+05
3.00E+05
2.00E+05
1.00E+05
0.00E+00
Total Cell Number
macromgel
pos.control(OCD)
Day 0
Day 7

FIGURE 44



Day 0

FIGURE 44 (CONTINUED)



Day 7

FIGURE 45

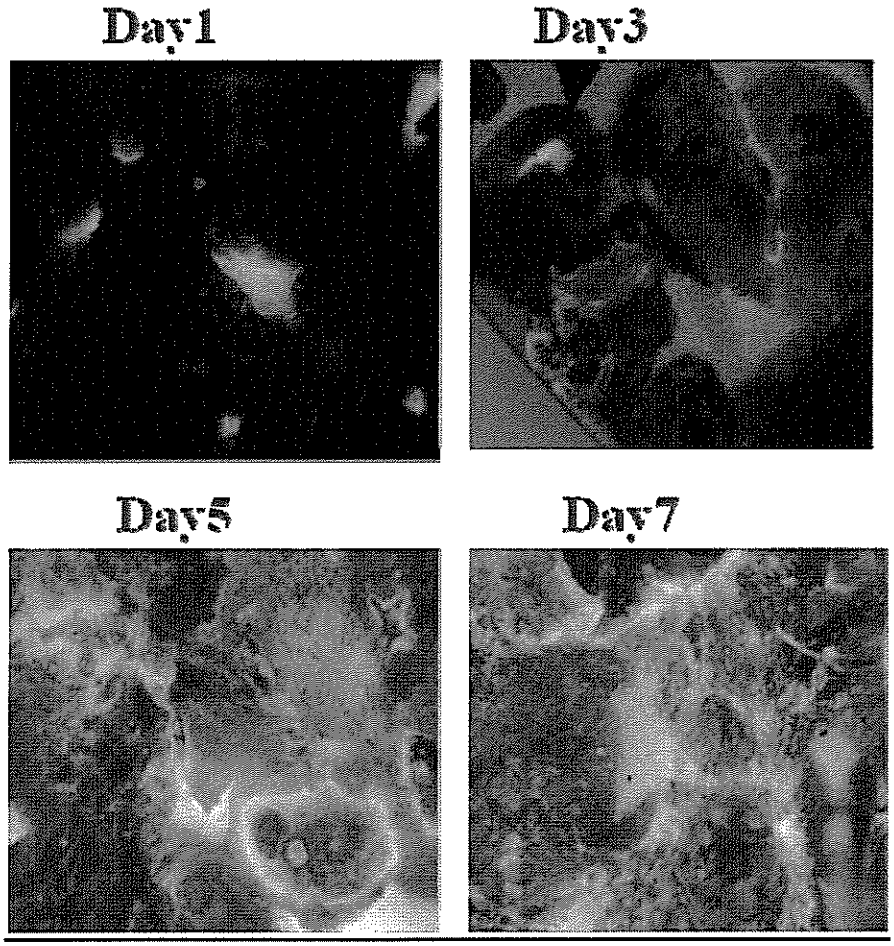


FIGURE 46A

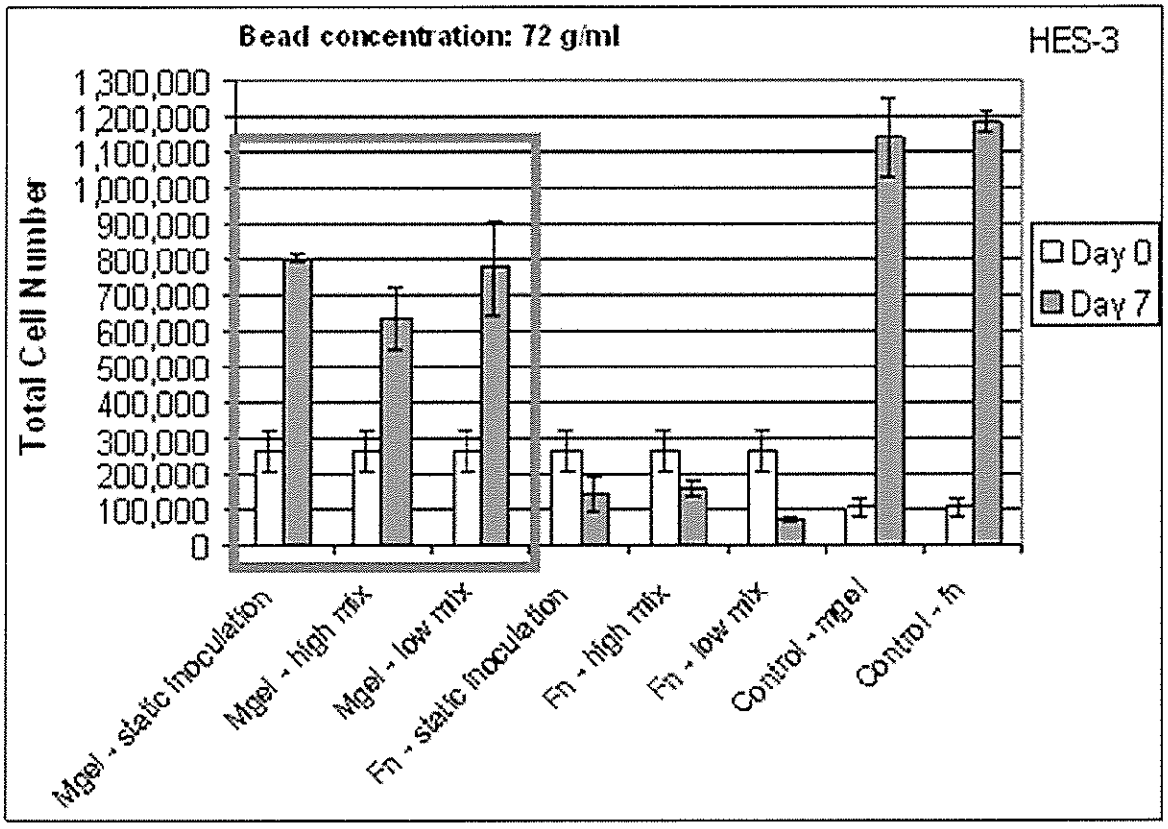

Bead concentration: 72 g/ml
HES-3
Total Cell Number
1,300,000
1,200,000
1,100,000
1,000,000
900,000
800,000
700,000
600,000
500,000
400,000
300,000
200,000
100,000
0
Day 0
Day 7
Mgel - static inoculation
Mgel - high mix
Mgel - low mix
Fn - static inoculation
Fn - high mix
Fn - low mix
Control - mgel
Control - fn

FIGURE 46B

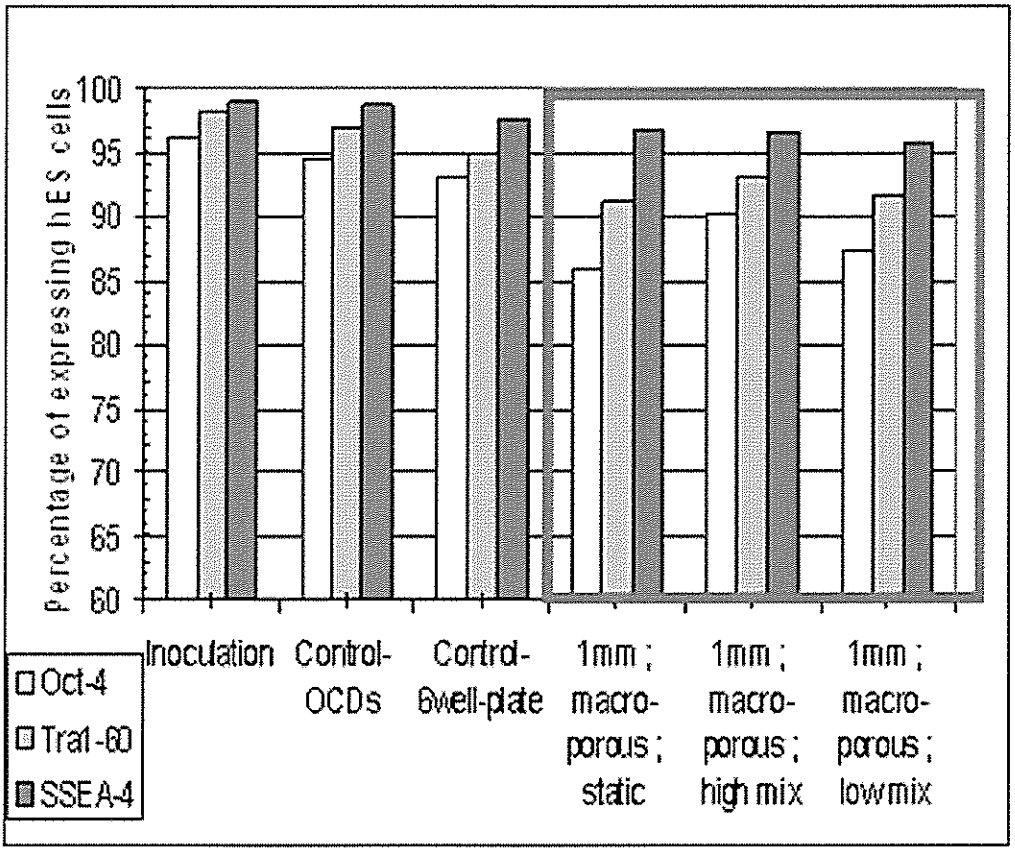

percentage of expressing hES cells
100
95
90
85
80
75
70
65
60
Inoculation
Control-OCDs
Control-6well-plate
1mm; macro-porous; static
1mm; macro-porous; high mix
1mm; macro-porous; low mix
Oct-4
Tra1-60
SSEA-4

FIGURE 47A

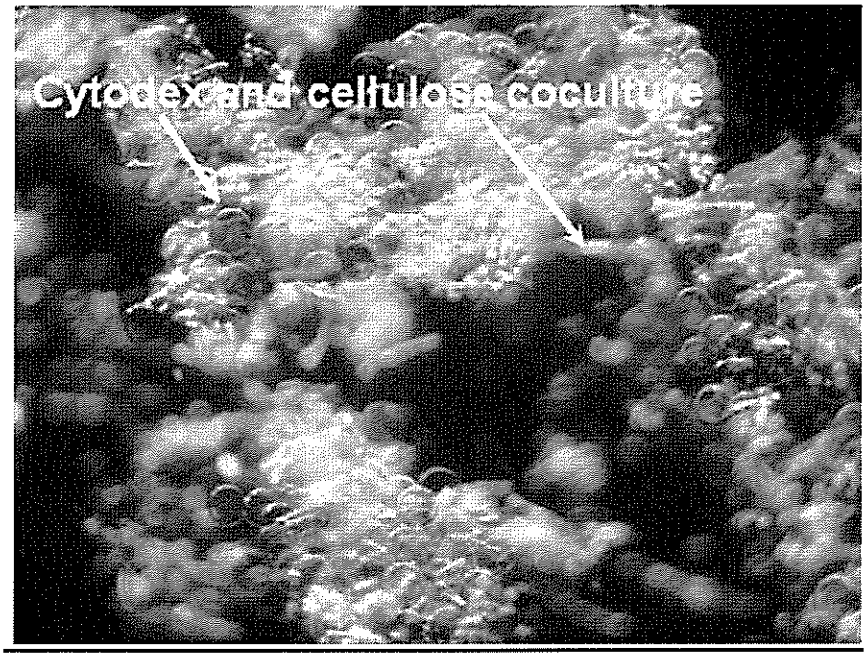


FIGURE 47B

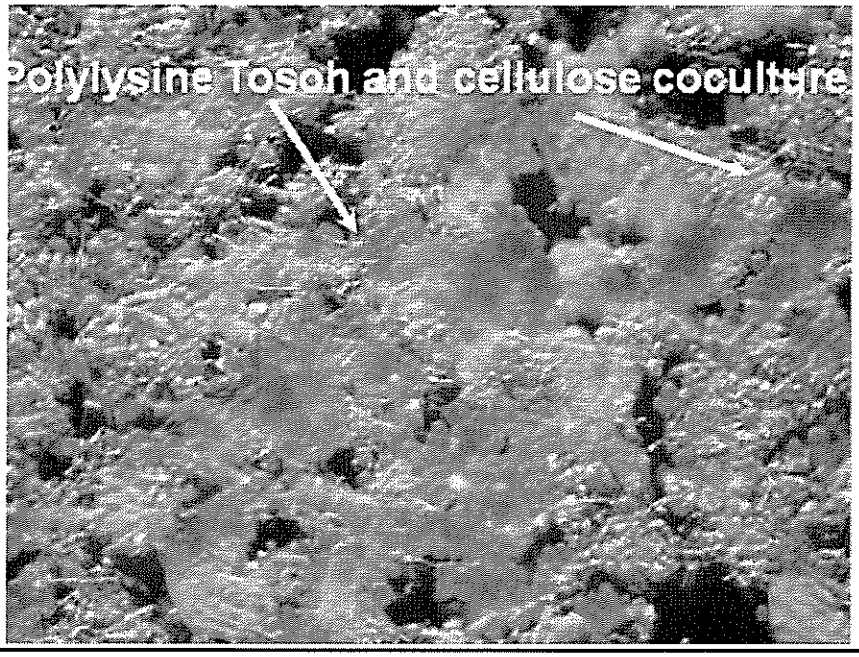


FIGURE 48A

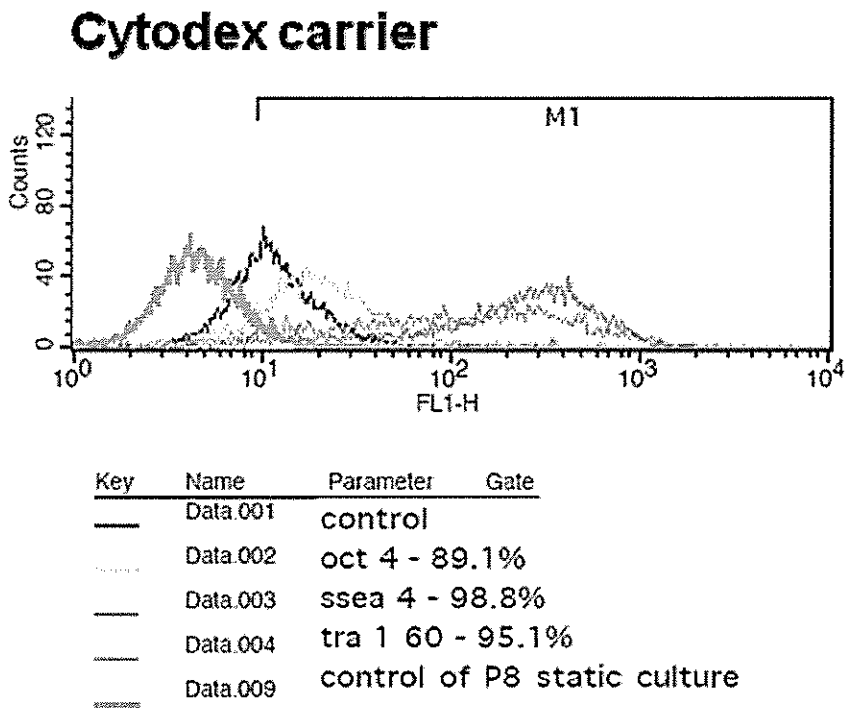
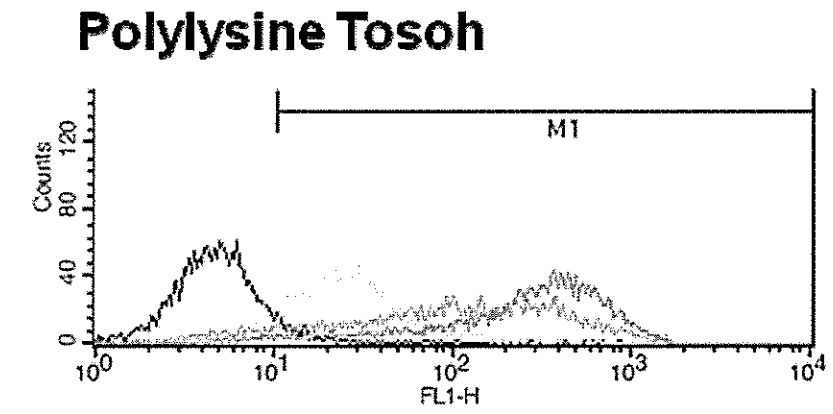

Cytodex carrier
M1
Counts
0
40
80
120
10^0
10^1
10^2
10^3
10^4
FL1-H
Key
Name
Parameter
Gate
Data.001
control
Data.002
oct 4 - 89.1%
Data.003
ssea 4 - 98.8%
Data.004
tra 1 60 - 95.1%
Data.009
control of P8 static culture

FIGURE 48B



Key	Name	Parameter	Gate
———	Data.005	control	
.....	Data.006	oct 4 - 84.8%	
———	Data.007	ssea 4 - 98.6%	
———	Data.008	tra 1 60 - 92.0%	

FIGURE 49

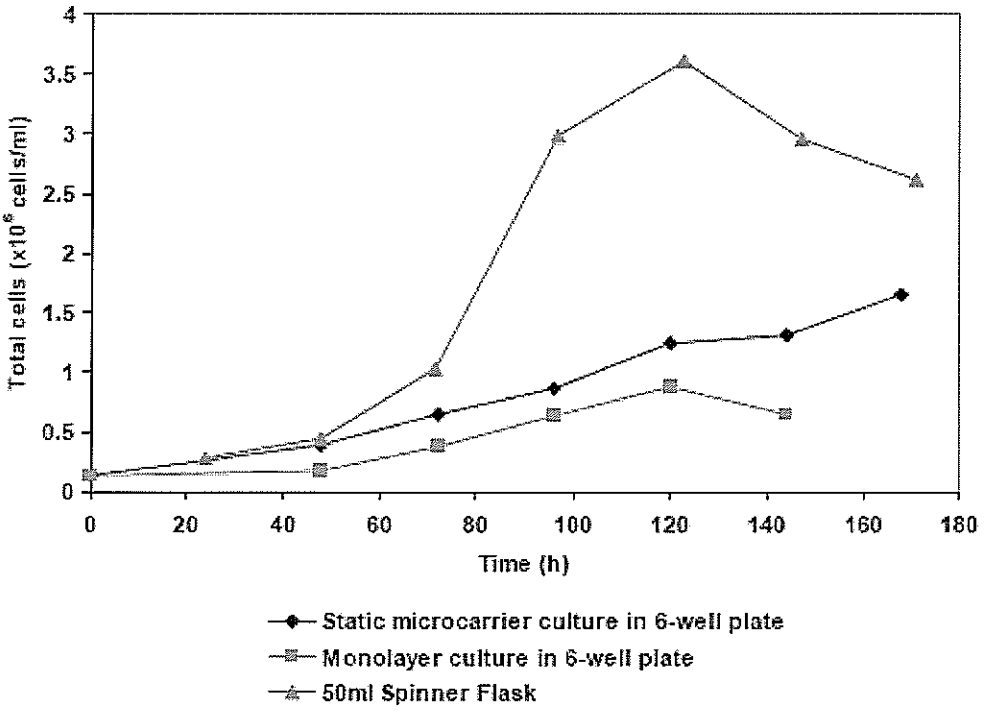

Total cells (x10^6 cells/ml)
Time (h)
Static microcarrier culture in 6-well plate
Monolayer culture in 6-well plate
50ml Spinner Flask

FIGURE 50



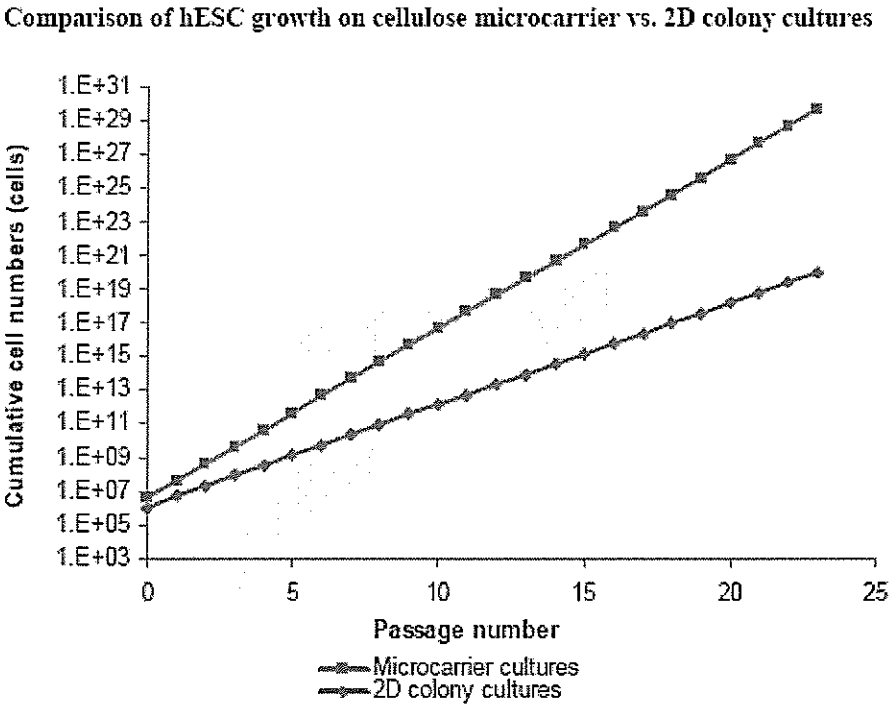

Comparison of hESC growth on cellulose microcarrier vs. 2D colony cultures
Cumulative cell numbers (cells)
1.E+31
1.E+29
1.E+27
1.E+25
1.E+23
1.E+21
1.E+19
1.E+17
1.E+15
1.E+13
1.E+11
1.E+09
1.E+07
1.E+05
1.E+03
0
5
10
15
20
25
Passage number
Microcarrier cultures
2D colony cultures

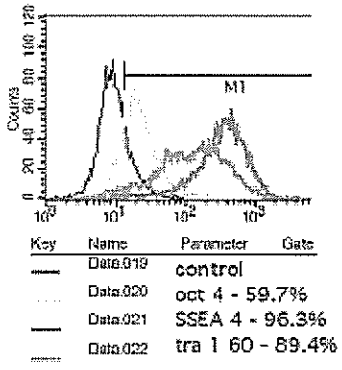
Figure 51

Passage 15 & 16 of hESC on matrigel coated DE53 carriers

Nuclei count

- P15= 9.4 E6 cells/well
- P16= 7.1 E6 cells/well

Passage 15



Passage 16

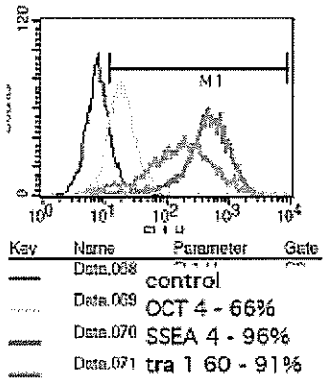
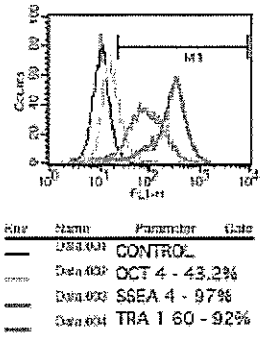
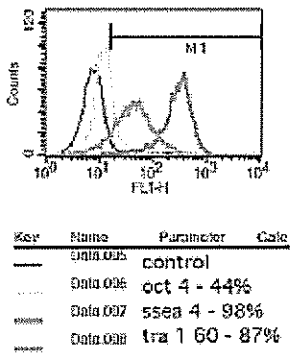


Figure 52

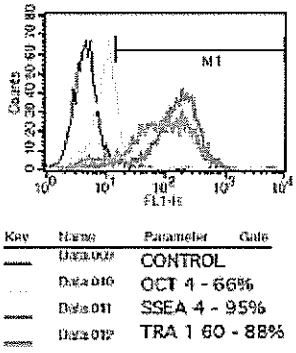
Passage 21



Passage 22



Passage 23



Replated Passage 23- P0 on 6cm

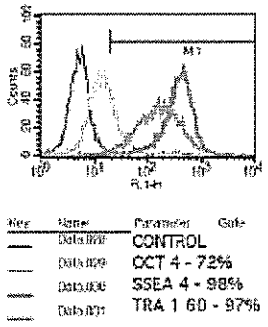
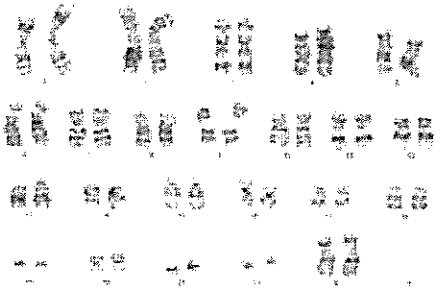


Figure 53

Karyotypes

HES-3

P22 on microcarriers



P25 on microcarriers

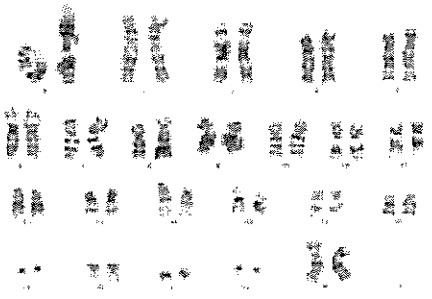


Figure 54

HES-2 P14 on microcarriers

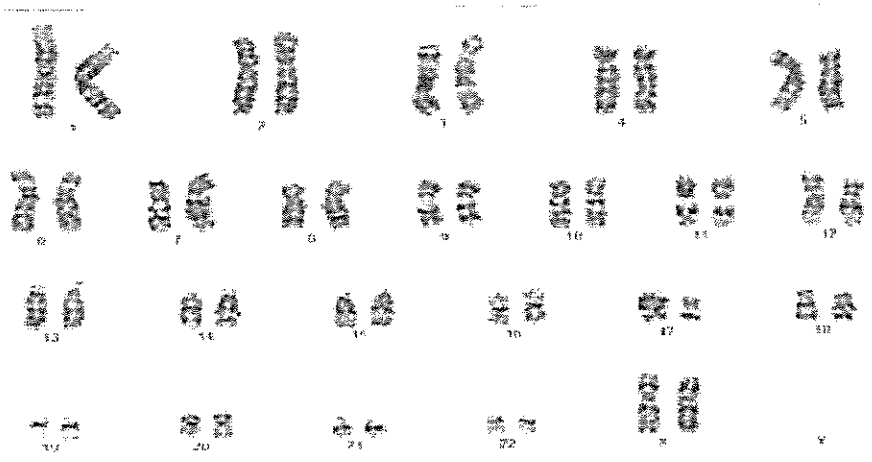


Figure 55

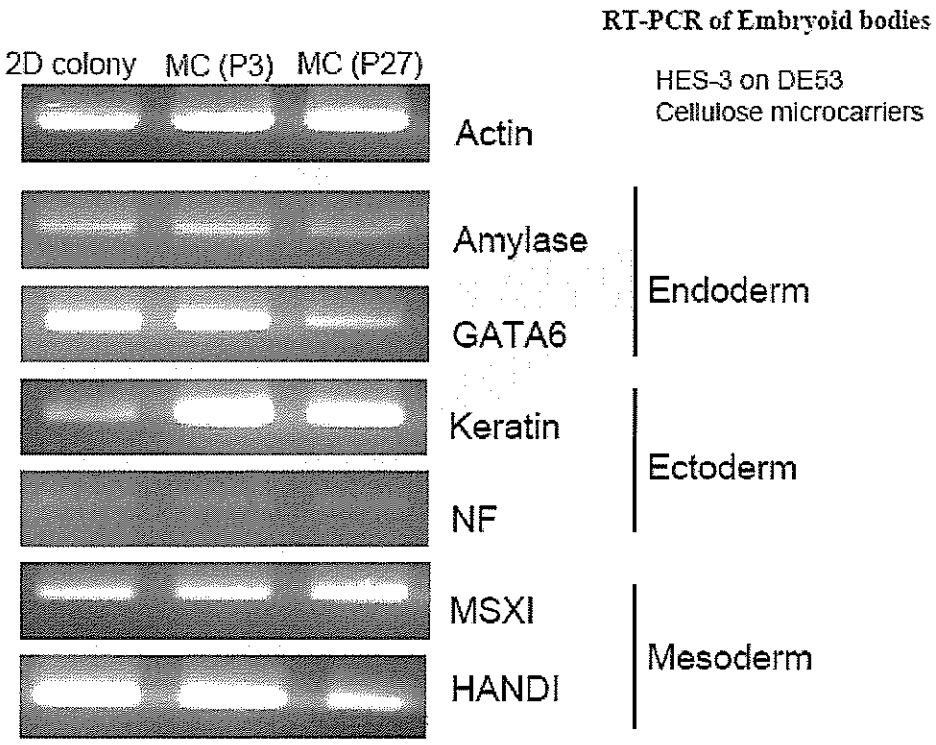


Figure 56

Teratomas

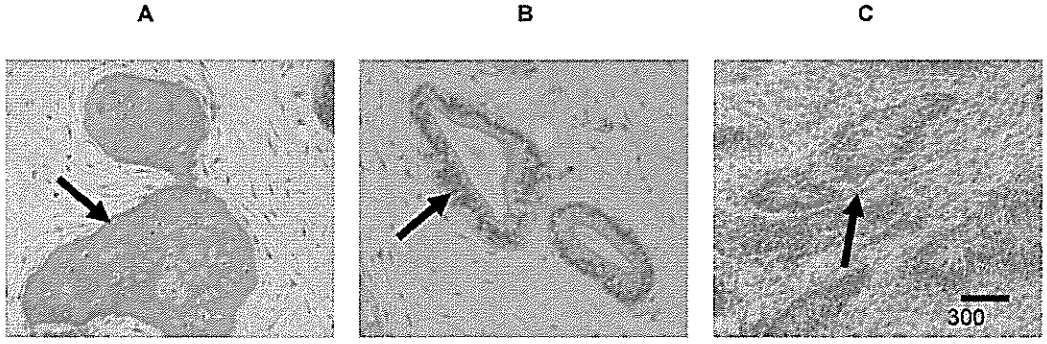


Figure 57

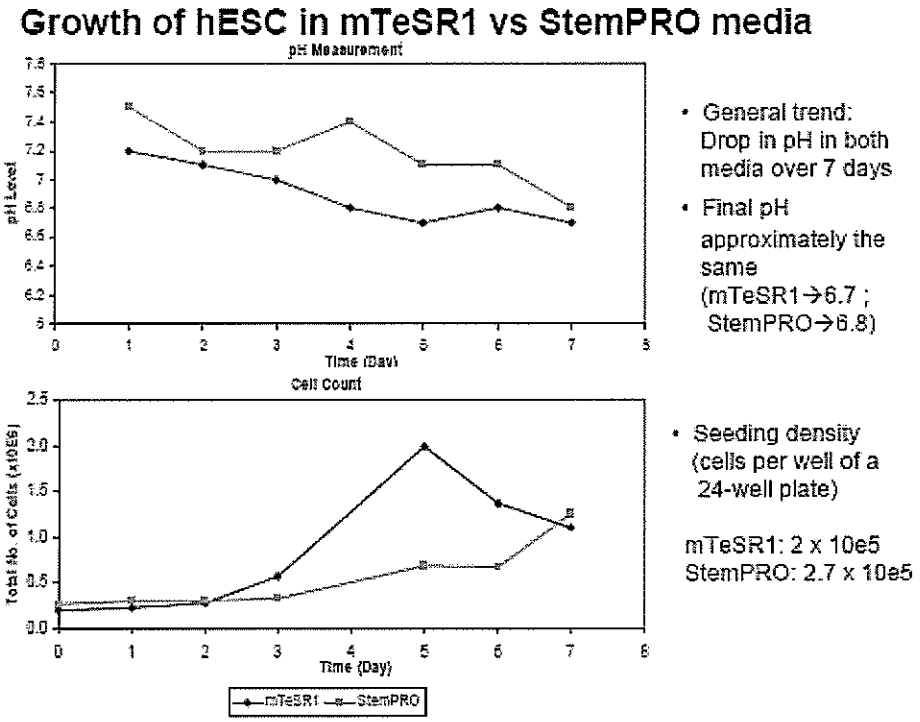
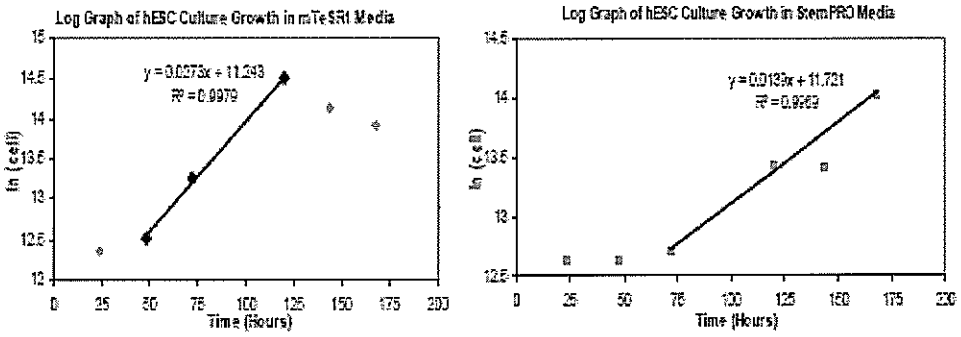

Growth of hESC in mTeSR1 vs StemPRO media
pH Measurement
pH Level
Time (Day)
Cell Count
Total No. of Cells (x10E5)
Time (Day)
mTeSR1
StemPRO
• General trend: Drop in pH in both media over 7 days
• Final pH approximately the same (mTeSR1→6.7 ; StemPRO→6.8)
• Seeding density (cells per well of a 24-well plate)
mTeSR1: 2 x 10e5
StemPRO: 2.7 x 10e5

Figure 58

Comparison of Doubling Time
(mTeSR1 vs StemPRO media)



	mTeSR1	StemPRO
Growth Rate, μ (h^{-1})	0.027	0.014
Doubling Time (h)	25	49

Figure 59

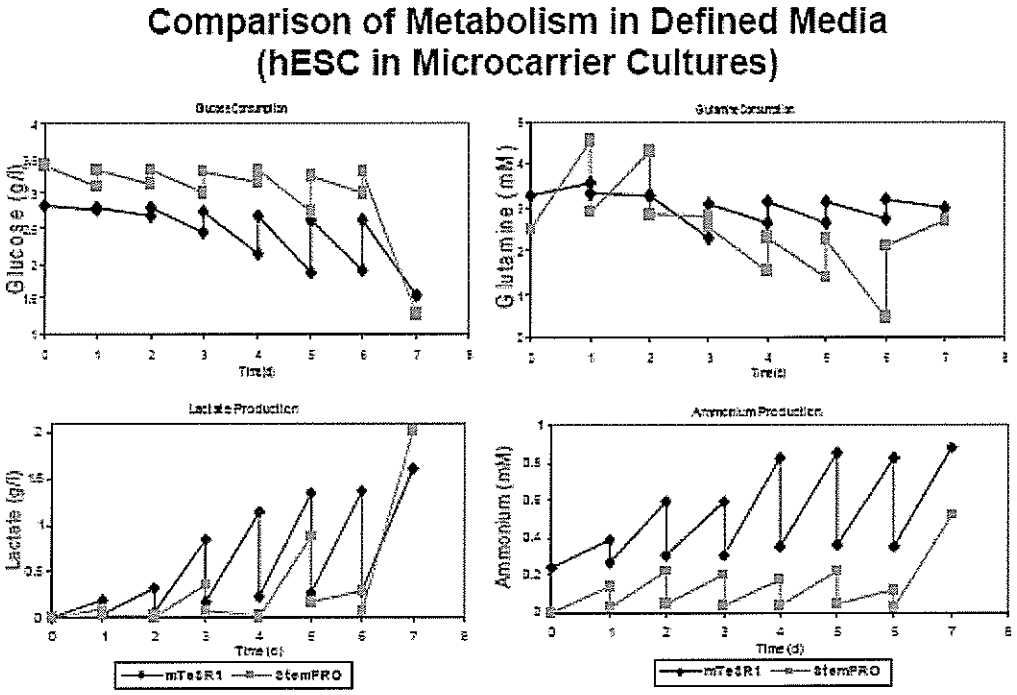

Comparison of Metabolism in Defined Media
(hESC in Microcarrier Cultures)
Glucose Consumption
Glucose (g/l)
Time(d)
Glutamine Consumption
Glutamine (mM)
Time(d)
Lactate Production
Lactate (g/l)
Time(d)
Ammonium Production
Ammonium (mM)
Time(d)
mTeSR1
StemPRO

Figure 60

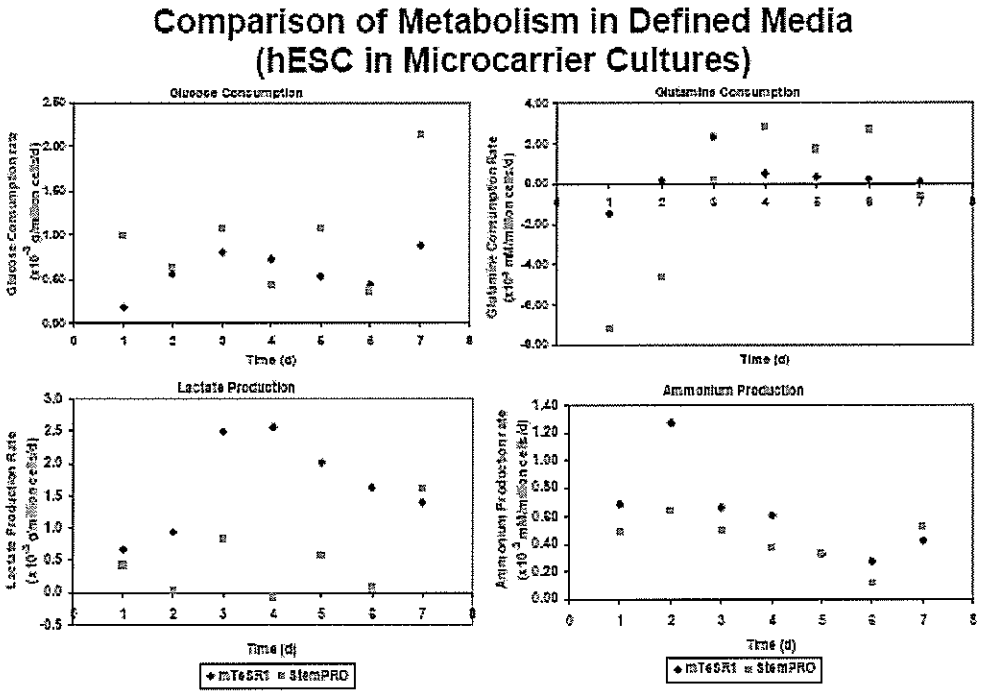

Comparison of Metabolism in Defined Media
(hESC in Microcarrier Cultures)
Glucose Consumption
Glutamine Consumption
Lactate Production
Ammonium Production
Time (d)
mTeSR1
StemPRO

Figure 61

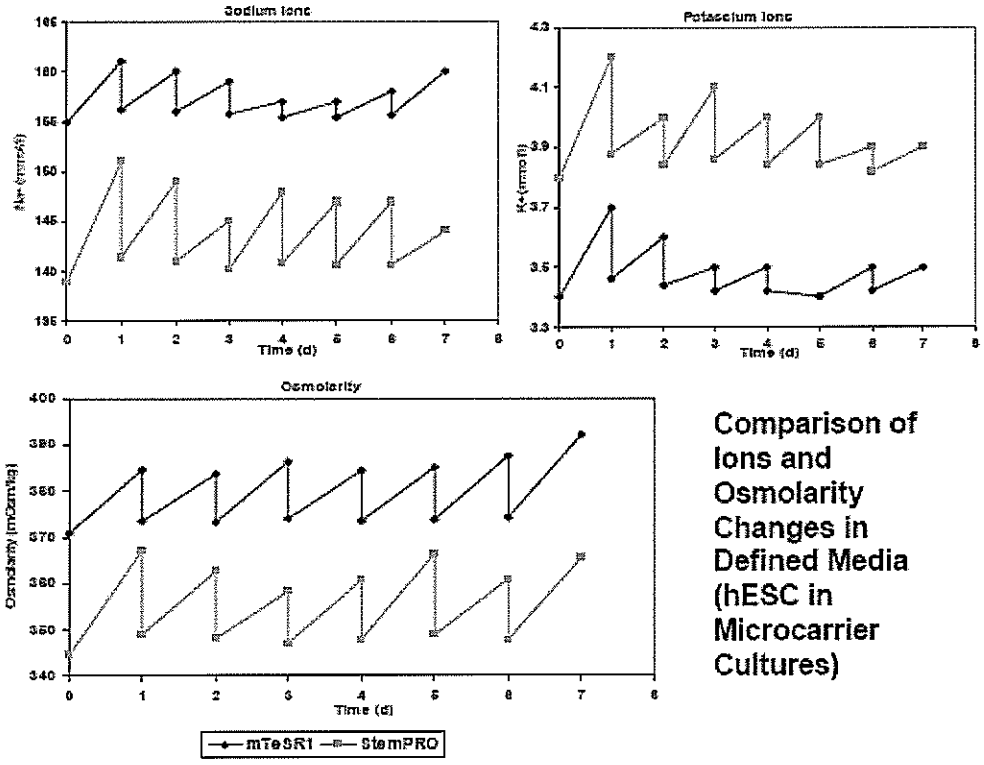

Sodium Ions
Potassium Ions
Osmolarity
Time (d)
mTeSR1
StemPRO
Comparison of Ions and Osmolarity Changes in Defined Media (hESC in Microcarrier Cultures)

Figure 62

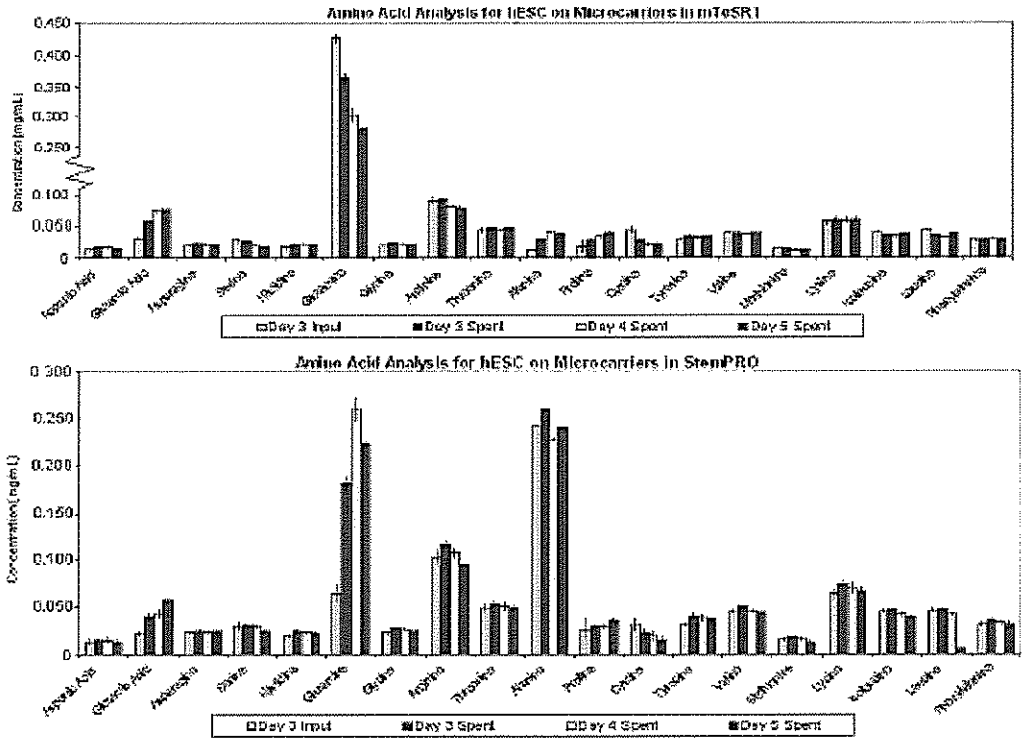

Amino Acid Analysis for hESC on Microcarriers in mTeSR1
Concentration (mg/mL)
0.450
0.400
0.350
0.300
0.250
0.100
0.050
0
Day 3 Input
Day 3 Spent
Day 4 Spent
Day 5 Spent
Amino Acid Analysis for hESC on Microcarriers in StemPRO
Concentration (mg/mL)
0.300
0.250
0.200
0.150
0.100
0.050
0
Day 3 Input
Day 3 Spent
Day 4 Spent
Day 5 Spent

Figure 63

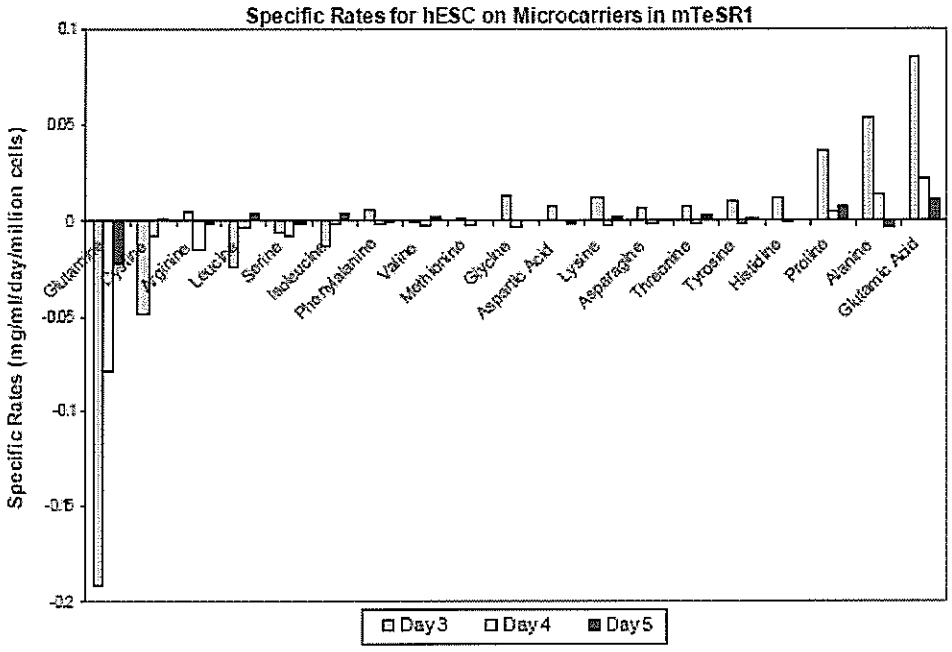


Figure 64a

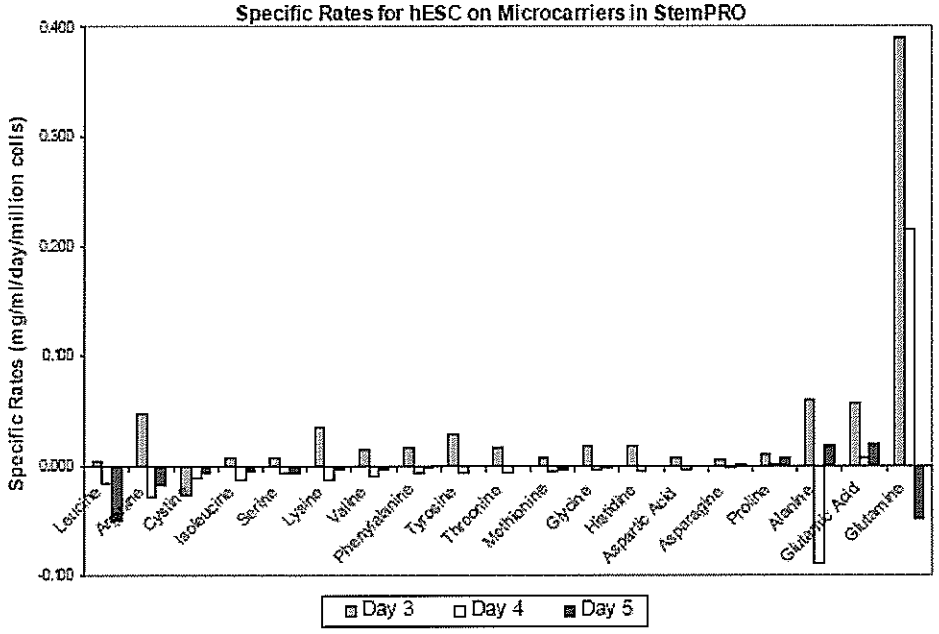


Figure 64b

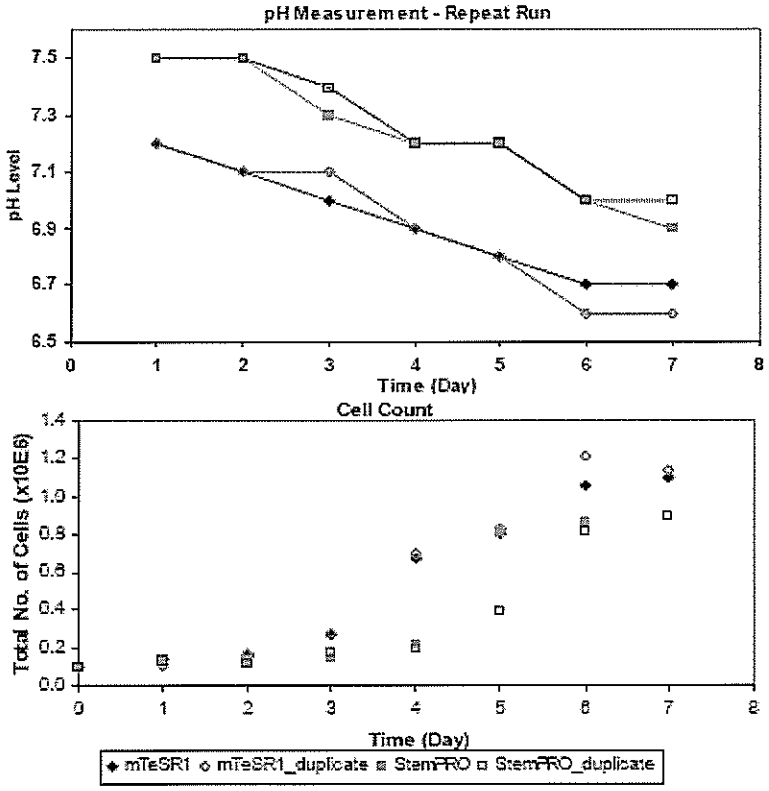
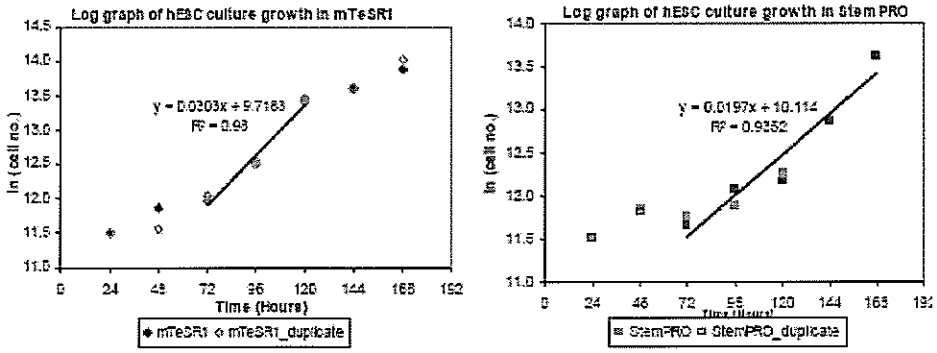

pH Measurement - Repeat Run
pH Level
Time (Day)
Cell Count
Total No. of Cells (x10E6)
Time (Day)
mTeSR1 mTeSR1_duplicate StemPRO StemPRO_duplicate
hESC on Microcarriers in Defined Media (Repeat Expt)

Figure 65

Growth Kinetics – Repeat Expt



	mTeSR1	StemPRO
Growth Rate, μ (h^{-1})	0.0303	0.0197
Doubling Time (h)	23	35

- mTeSR1 → Better growth kinetics

Figure 66

HES-3 Cell Growth

Comparison between different runs and platforms

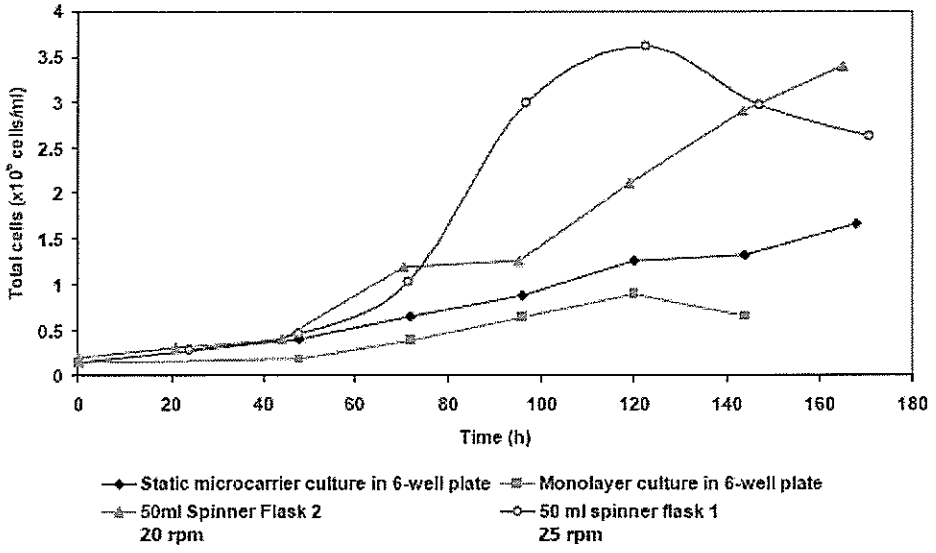


Figure 67

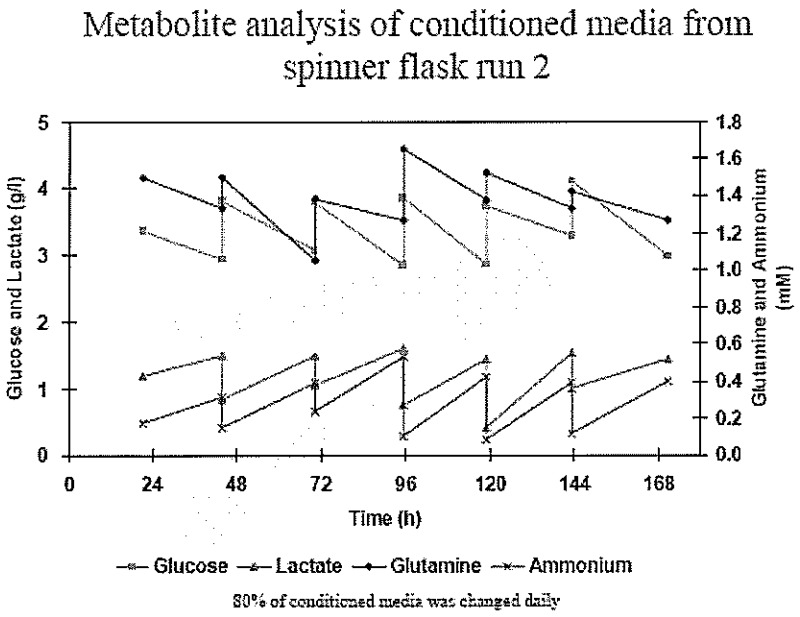


Figure 68

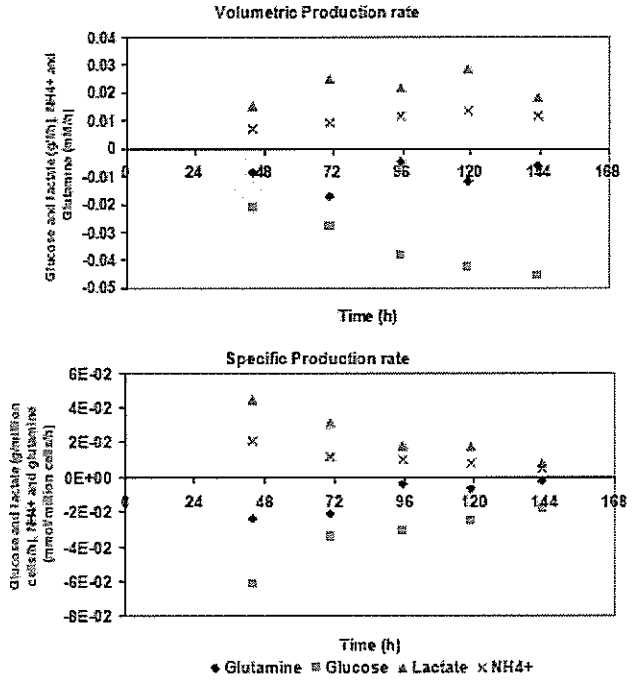


Figure 69

pH and osmolarity conditions in spinner flask 2

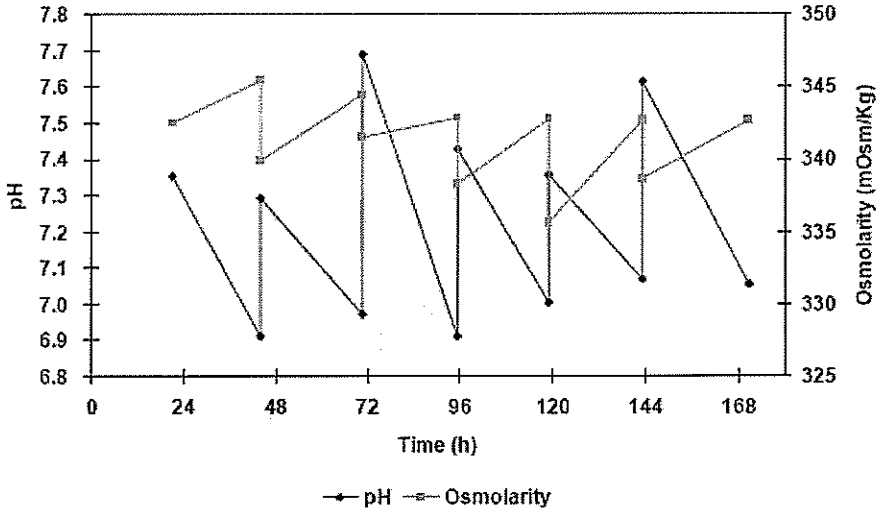


Figure 70

Pluripotent markers analysis of HES-3 culture on microcarriers in spinner flask 2

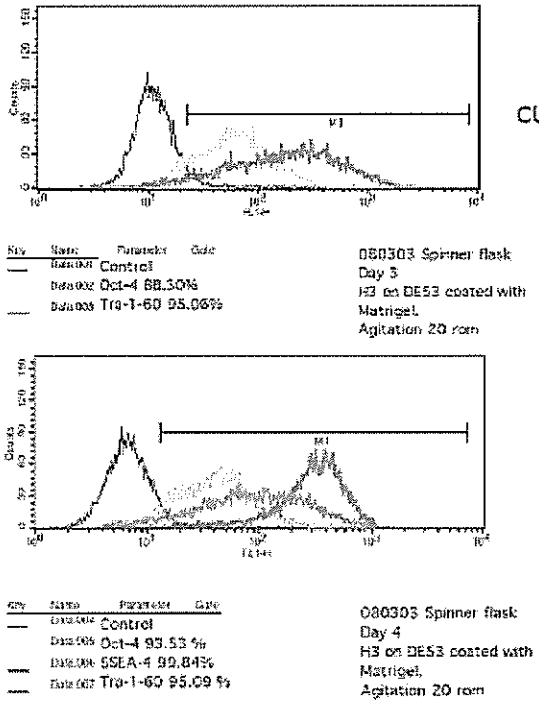


Figure 71

Observations of HES-3 in spinner flask 2 cultures

Day 4 Day 5

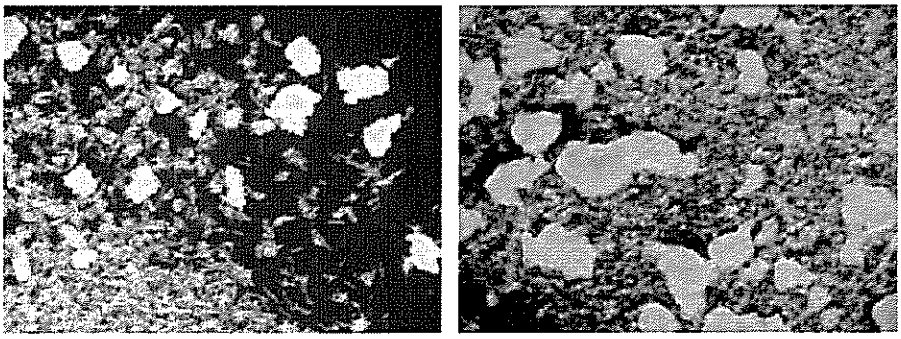


Figure 72

HES-2 Growth Curve in Spinner Flask Culture in Conditioned Media

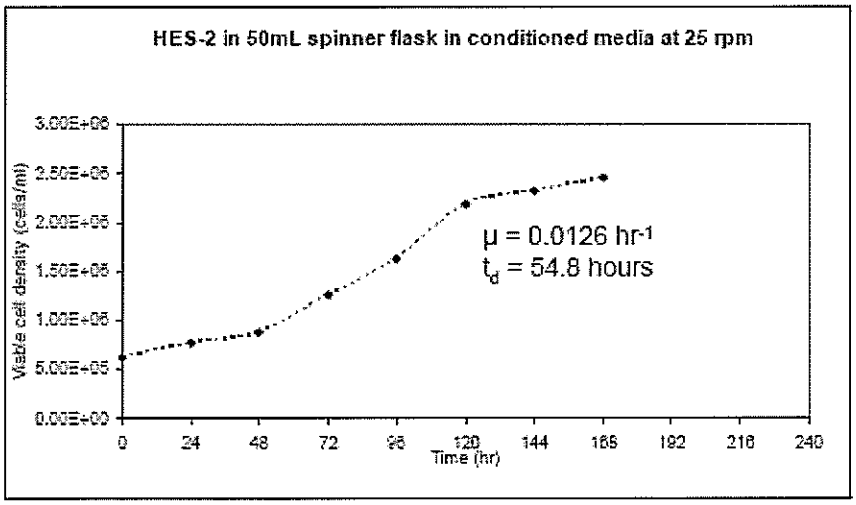


Figure 73

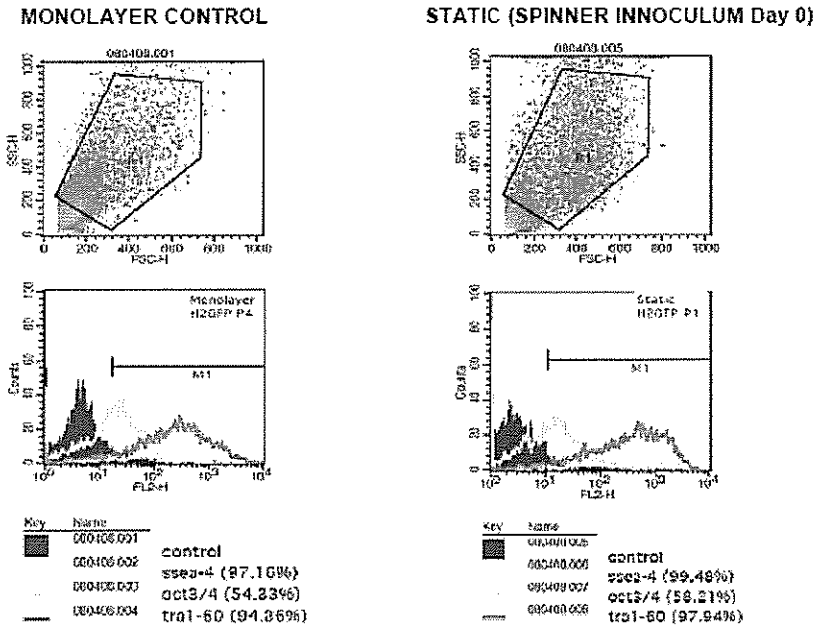


Figure 74

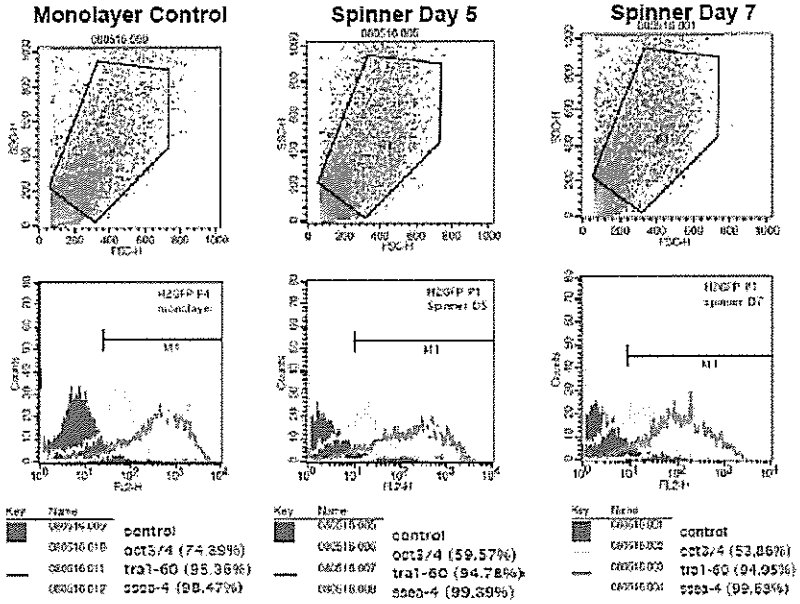


Figure 75

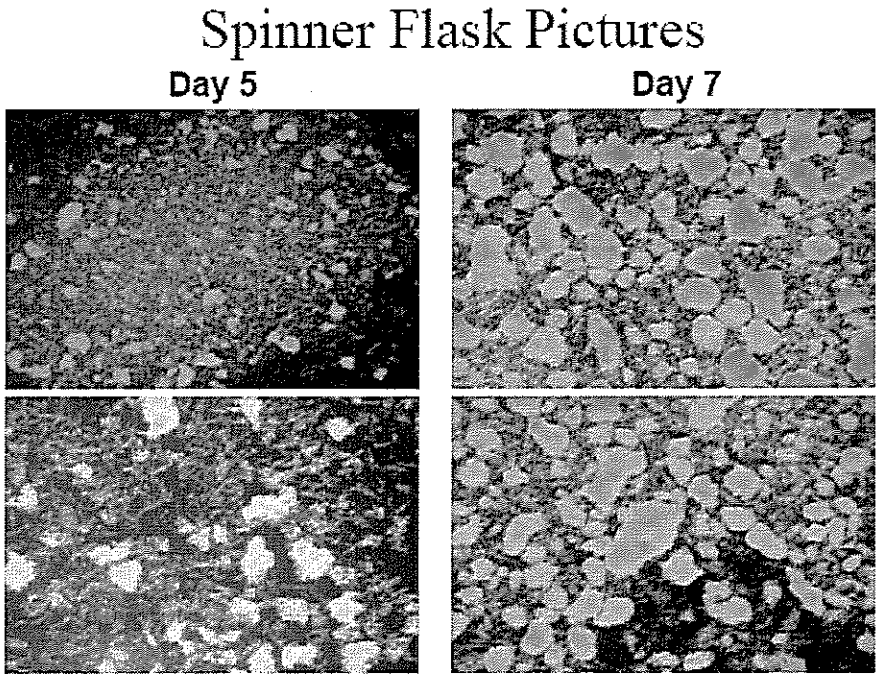


Figure 76

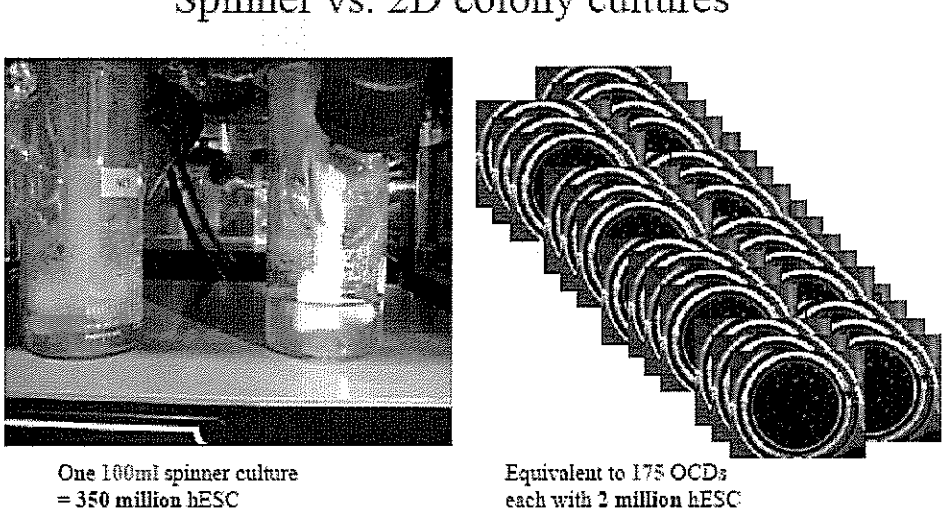


Figure 77

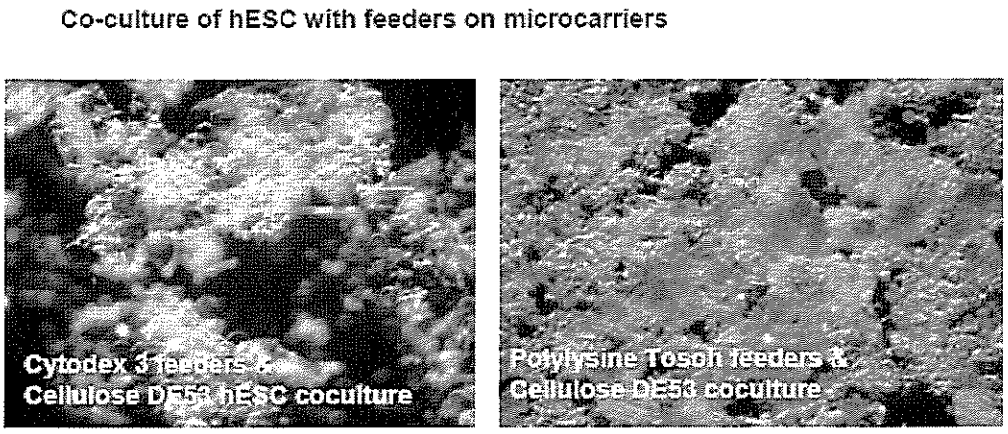


Figure 78

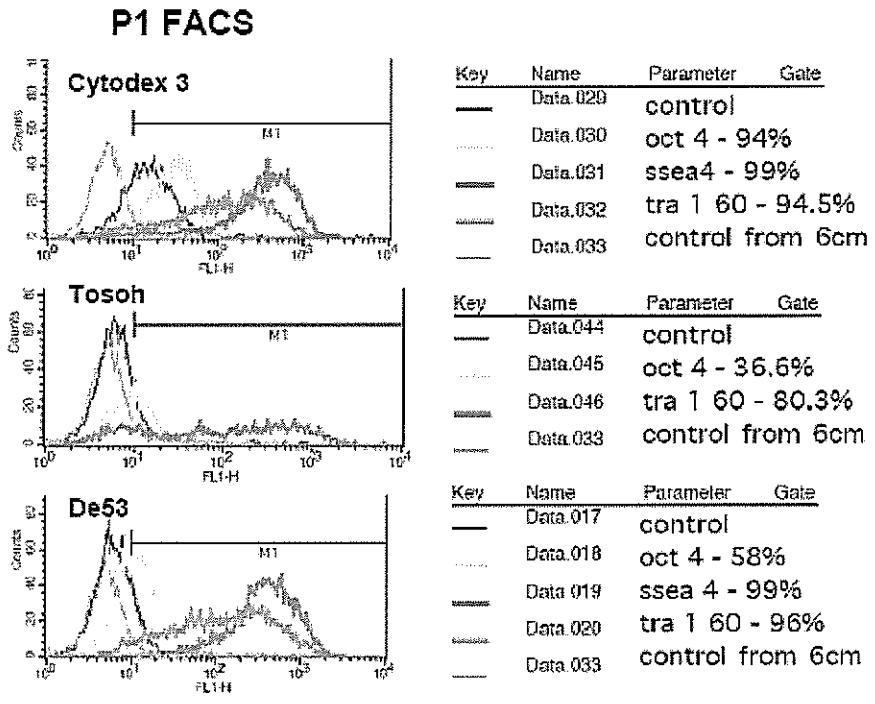


Figure 79

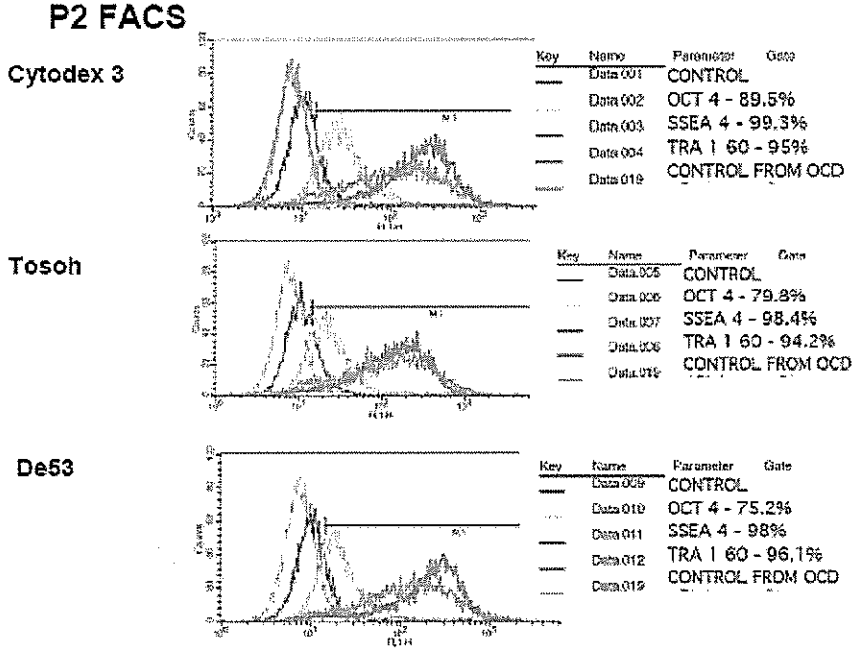


Figure 80

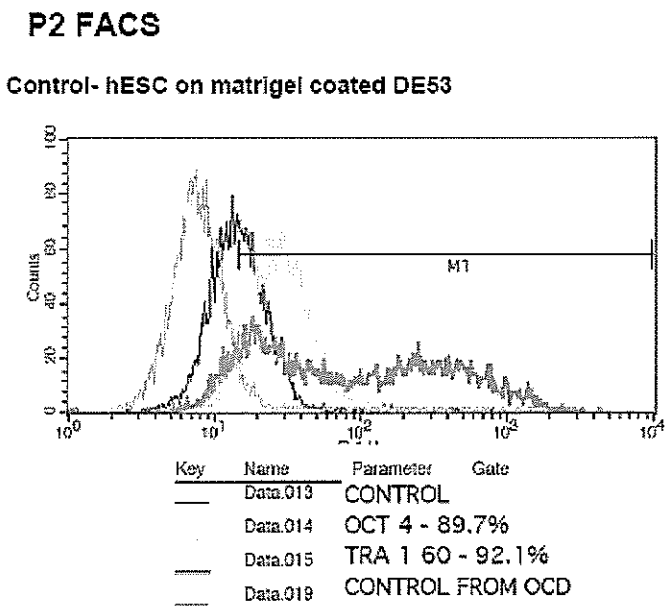


Figure 81

Passage 1

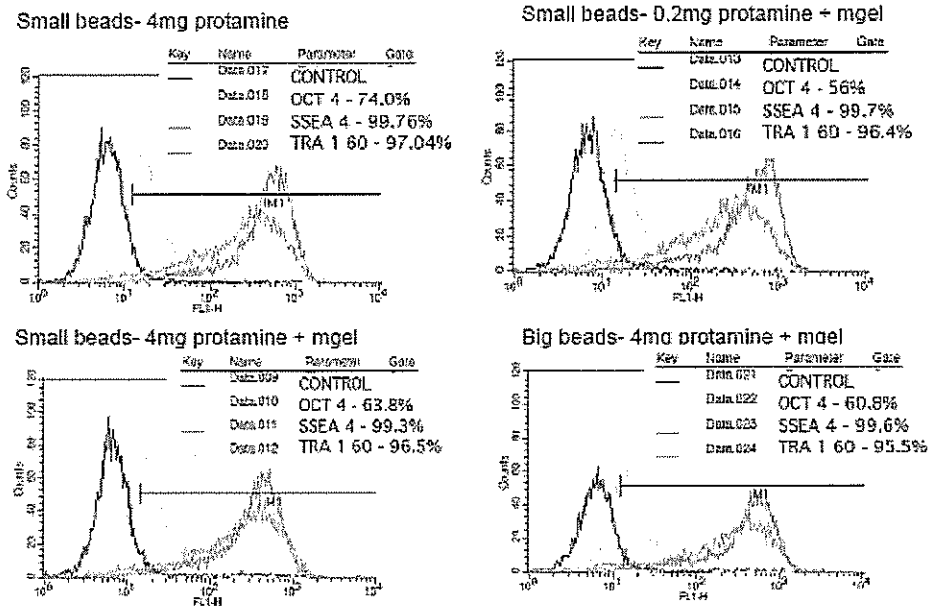


Figure 82

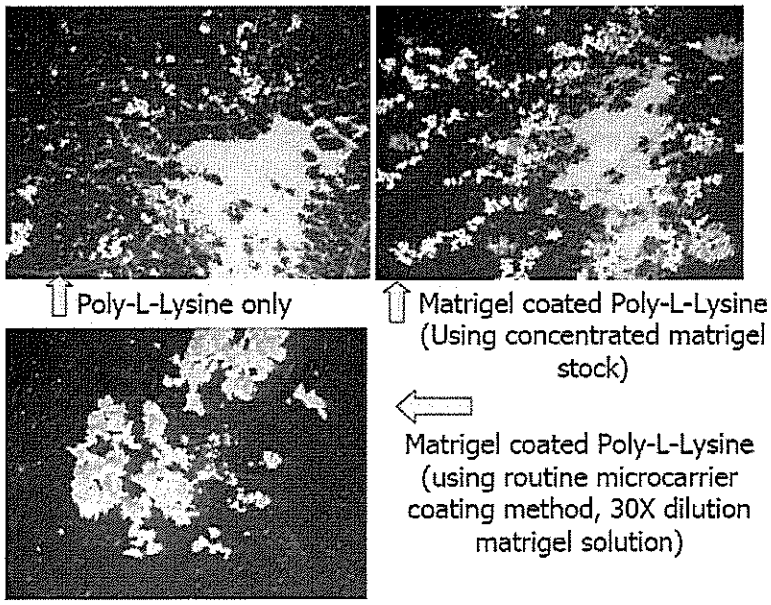


Figure 83

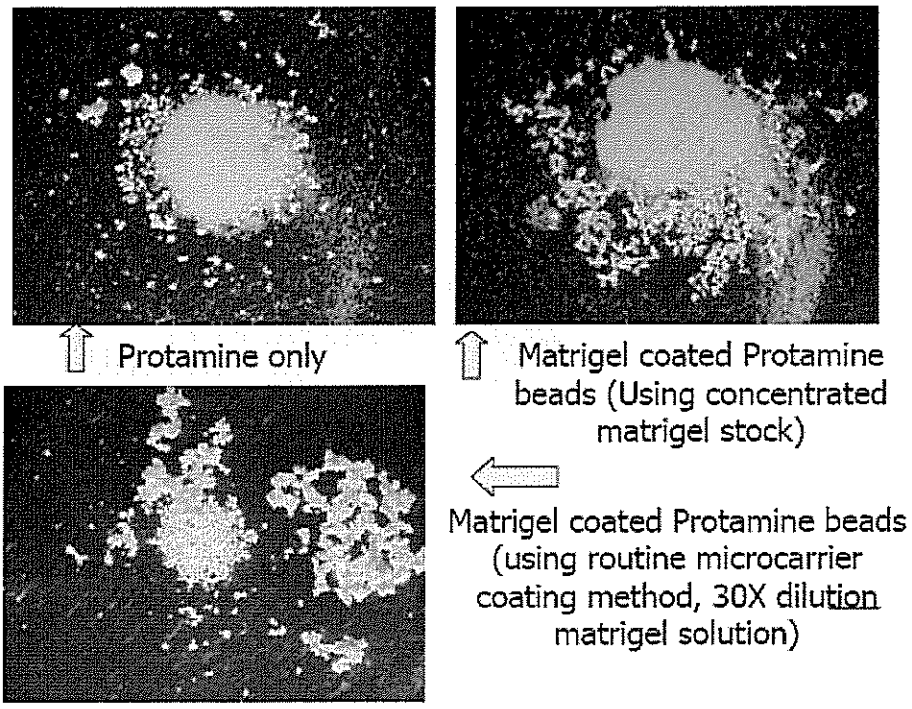


Figure 84

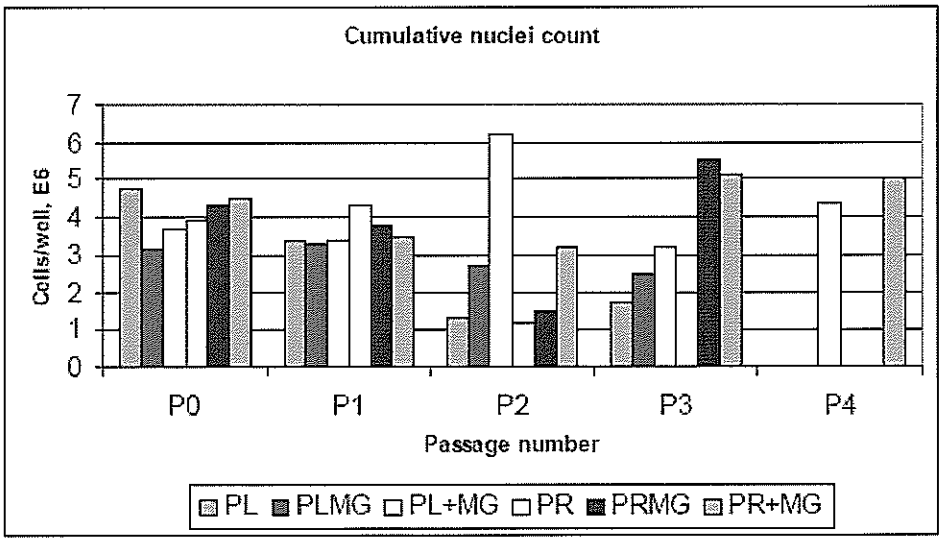
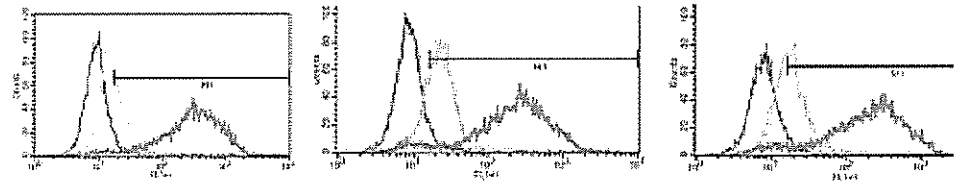


Figure 85

FACS for P1

PL **PLMG** **PL+MG**



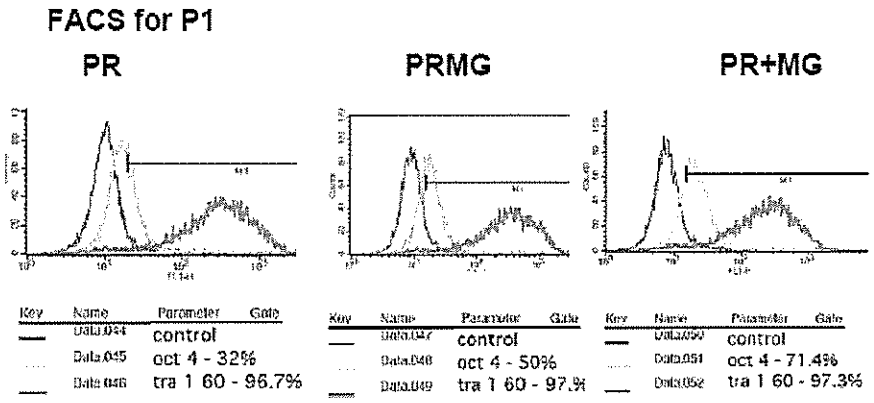
Key	Name	Parameter	Gate
	Data.038	control	
	Data.039	oct 4 - 32%	
	Data.040	tra 1 60 - 97.1%	

Key	Name	Parameter	Gate
	Data.035	control	
	Data.036	oct 4 - 75.4%	
	Data.037	tra 1 60 - 94.7%	

Key	Name	Parameter	Gate
	Data.041	control	
	Data.042	oct 4 - 52.7%	
	Data.043	tra 1 60 - 94.4%	

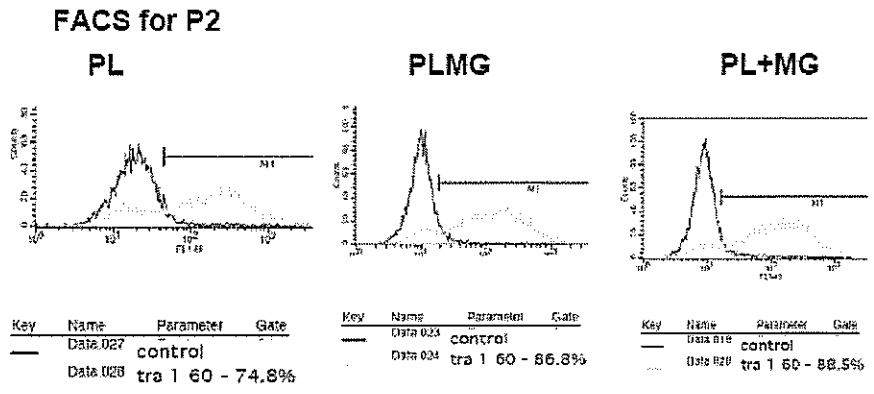
PL	PLMG	PL+MG	PR	PRMG	PR+MG
Poly-lysine	Polylysine coupled with matrigel	Poly lysine coated with matrigel	Protamine	Protamine coupled with matrigel	Protamine coated with matrigel

Figure 86



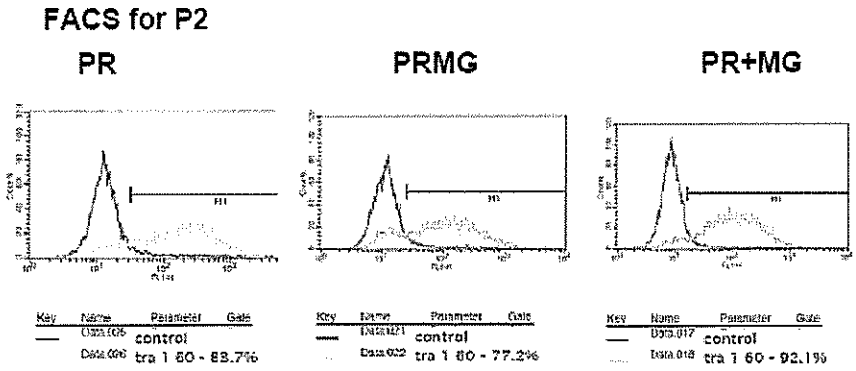
PL	PLMG	PL+MG	PR	PRMG	PR+MG
Poly-lysine	Polylysine coupled with matrigel	Poly lysine coated with matrigel	Protamine	Protamine coupled with matrigel	Protamine coated with matrigel

Figure 87



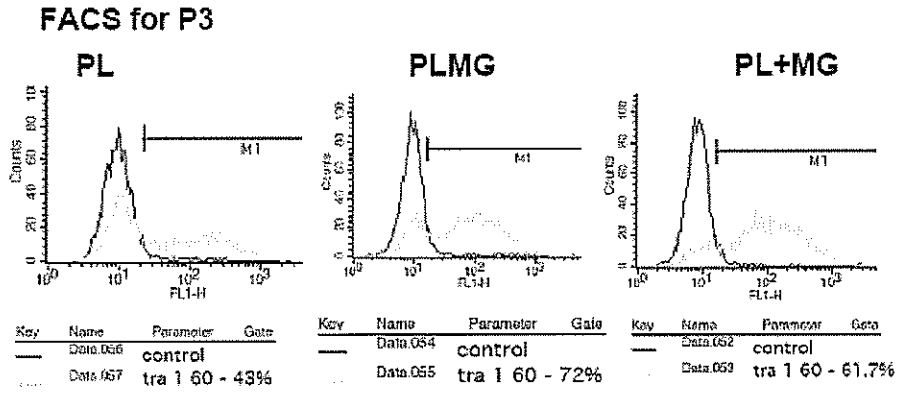
PL	PLMG	PL+MG	PR	PRMG	PR+MG
Poly-lysine	Polylysine coupled with matrigel	Poly lysine coated with matrigel	Protamine	Protamine coupled with matrigel	Protamine coated with matrigel

Figure 88



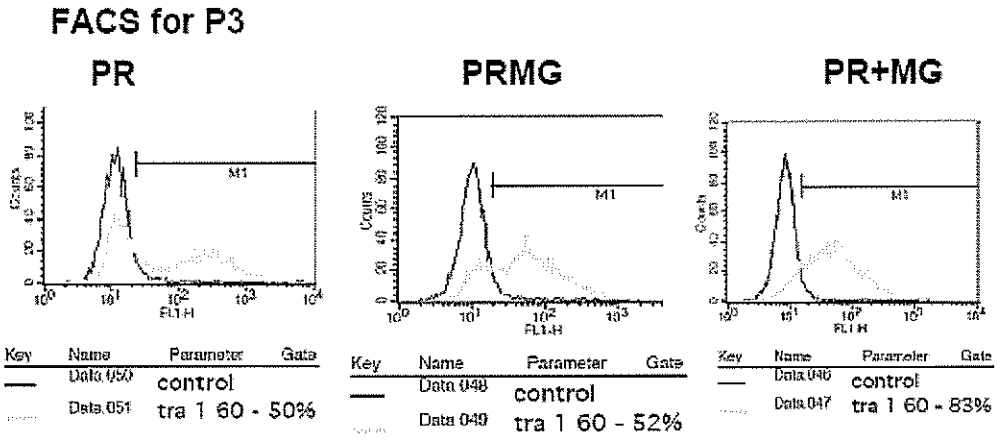
PL	PLMG	PL+MG	PR	PRMG	PR+MG
Poly-lysine	Polylysine coupled with matrigel	Poly lysine coated with matrigel	Protamine	Protamine coupled with matrigel	Protamine coated with matrigel

Figure 89



PL	PLMG	PL+MG	PR	PRMG	PR+MG
Poly-lysine	Polylysine coupled with matrigel	Poly lysine coated with matrigel	Protamine	Protamine coupled with matrigel	Protamine coated with matrigel

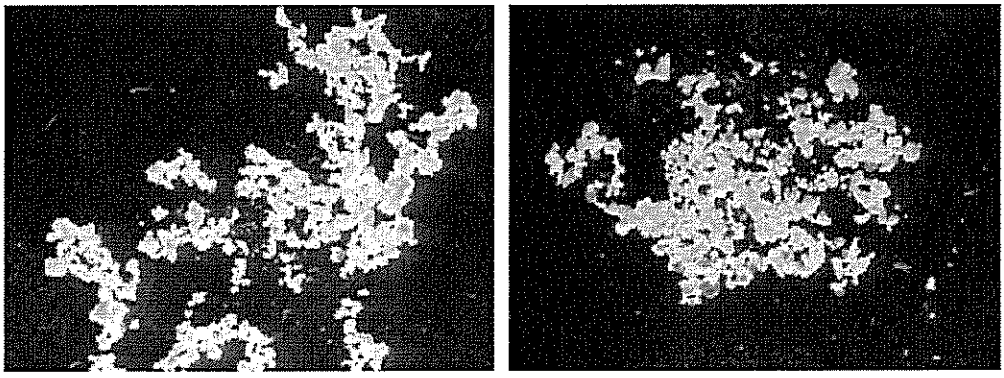
Figure 90



PL	PLMG	PL+MG	PR	PRMG	PR+MG
Poly-lysine	Polylysine coupled with matrigel	Poly lysine coated with matrigel	Protamine	Protamine coupled with matrigel	Protamine coated with matrigel

Figure 91

Passage 4



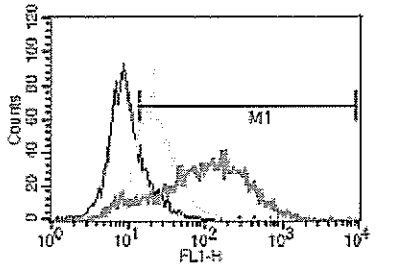
Tosoh bead coated with Poly-L-Lysine and coated with Matrigel

Tosoh bead coated with Protamine and coated with Matrigel

Figure 92

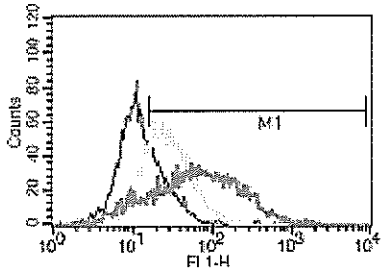
FACS for P4

PL+MG



Key	Name	Parameter	Gate
—	Data.013	control	
·····	Data.014	oct 4 - 62%	
———	Data.015	tra 1 60 - 78%	

PR+MG



Key	Name	Parameter	Gate
—	Data.016	control	
·····	Data.017	oct 4 - 46%	
———	Data.018	tra 1 60 - 59%	

PL÷MG	PR+MG
Poly lysine coated with matrigel	Protamine coated with matrigel

Figure 93

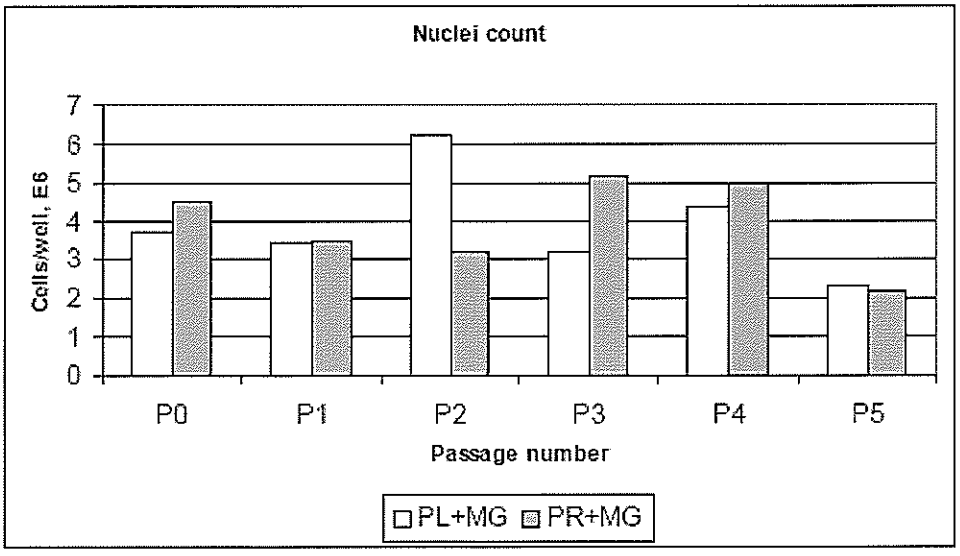
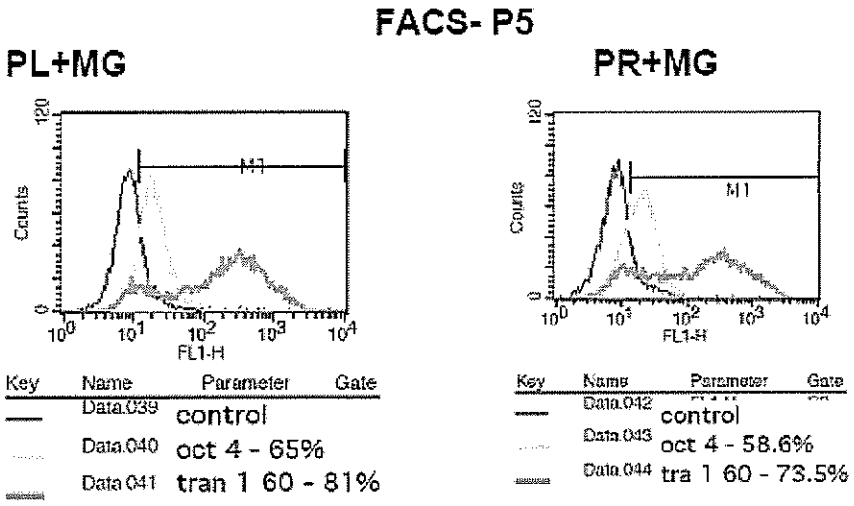


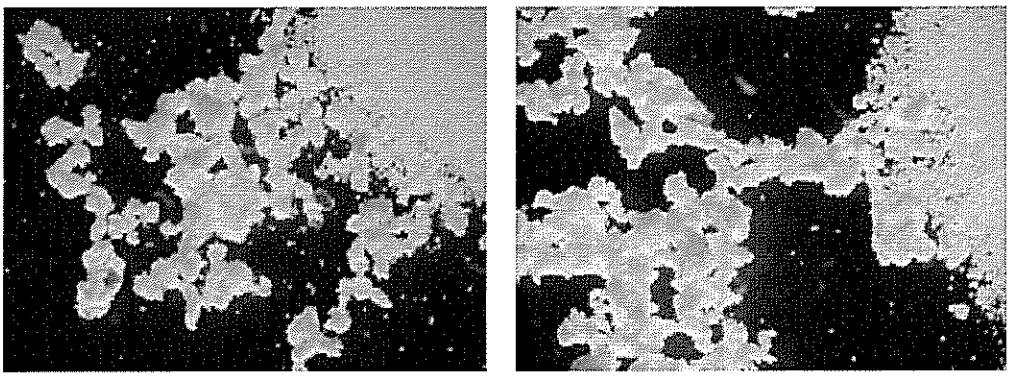
Figure 94



PL+MG	PR+MG
Poly lysine coated with matrigel	Protamine coated with matrigel

Figure 95

Passage 5



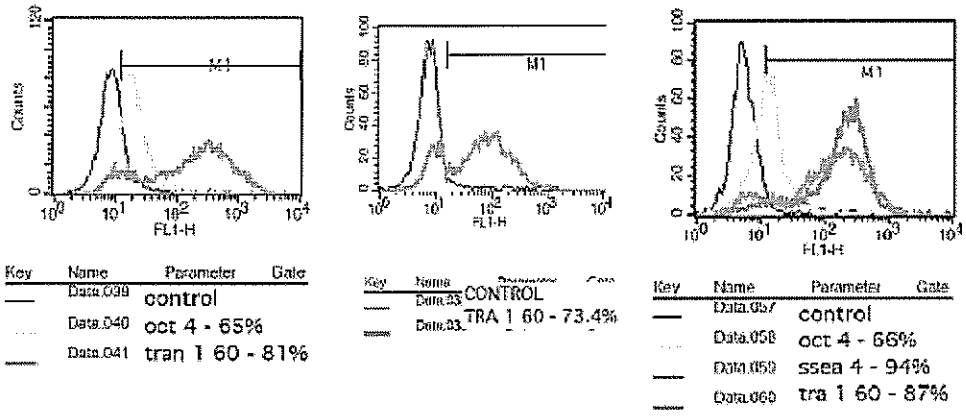
Tosoh beads coated with Poly-L-Lysine+Matrigel

Tosoh beads coated with Protamine+Matrigel

Figure 96

FACS- PL+MG

P5 **P6** **P7**

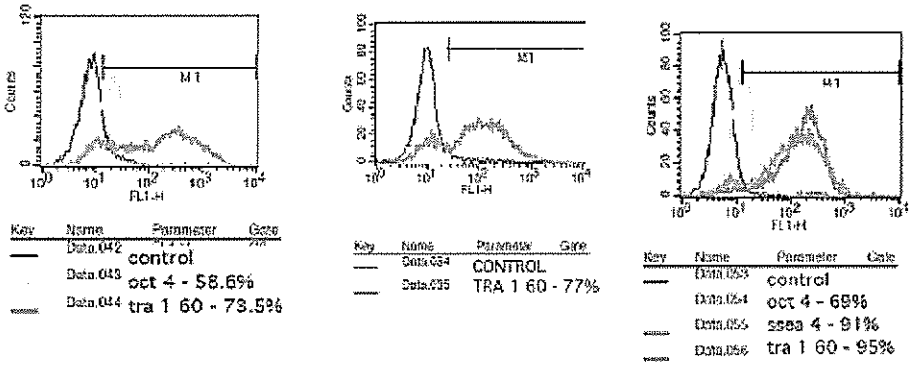


Note: main focus of experiment at this stage was to optimise no of beads to cells. "Optimise at 48,000 beads to around seeding of 1E6 cells per well

Figure 97

FACS- PR+MG

P5 **P6** **P7**



Note: main focus of experiment at this stage was to optimise no of beads to cells. "Optimise at 48,000 beads to around seeding of 1E6 cells per well

Figure 98

hESC on matrigel coated and uncoated Cytodex 3 microcarriers

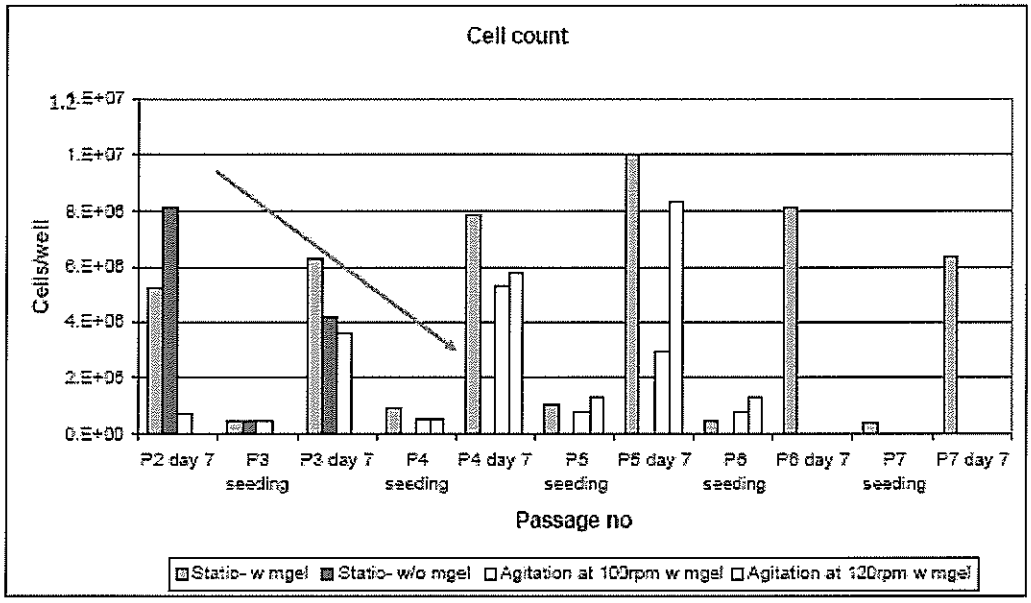


Figure 99

hESC on matrigel coated Cytodex 3 microcarriers

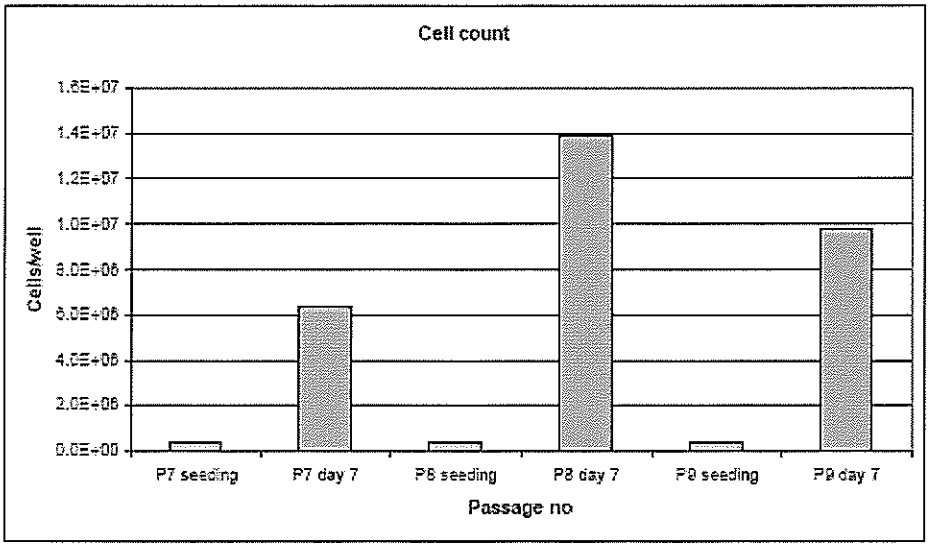
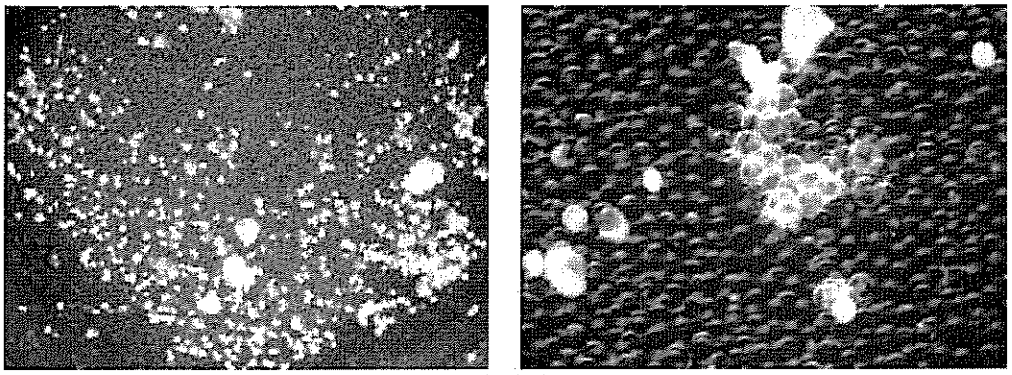
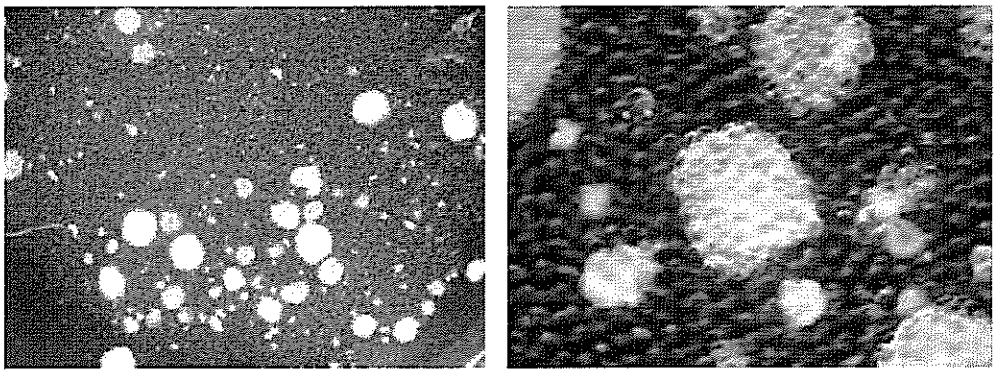


Figure 100



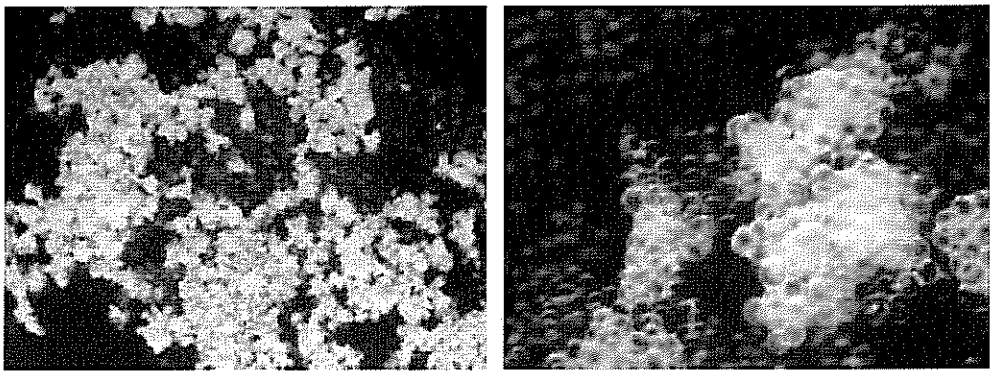
Cytodex 3 without Matrigel, non-agitated

Figure 101



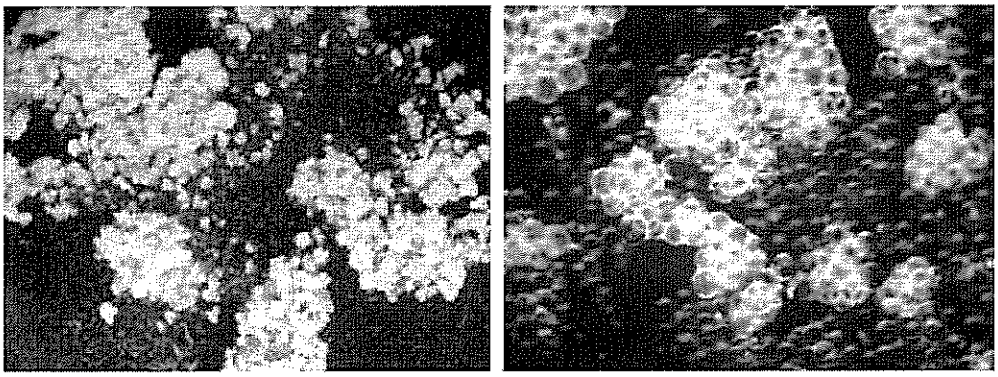
Cytodex 3 without Matrigel agitated at 100 rpm

Figure 102



Cytodex 3 coated with Matrigel, non-agitated

Figure 103



Cytodex 3 coated with Matrigel agitated at 100 rpm

Figure 104

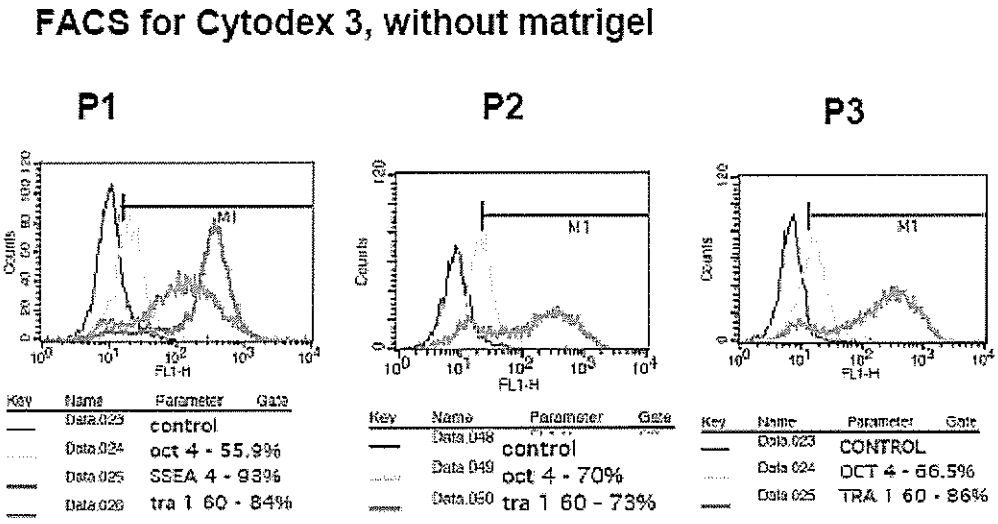


Figure 105

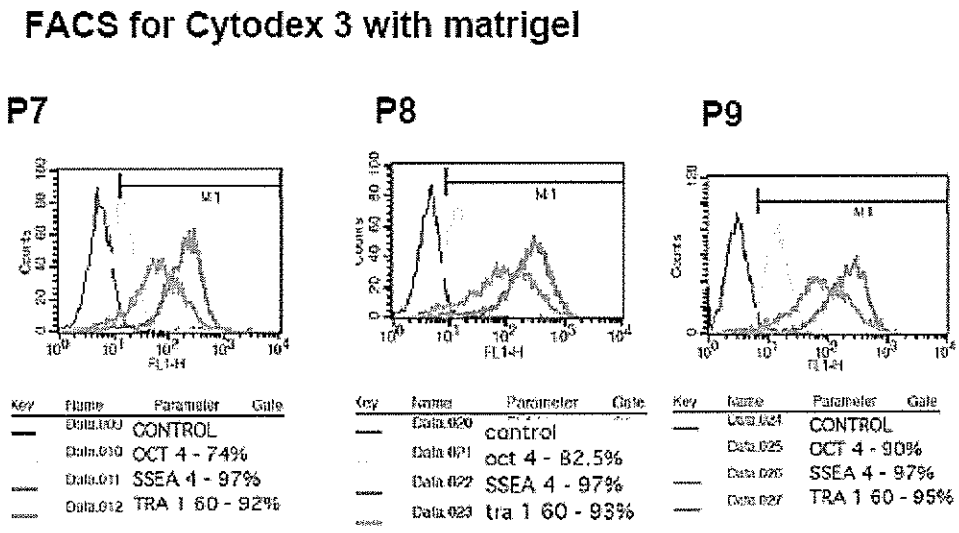


Figure 106

FACS for Cytodex 3 without matrigel with agitation at 100rpm

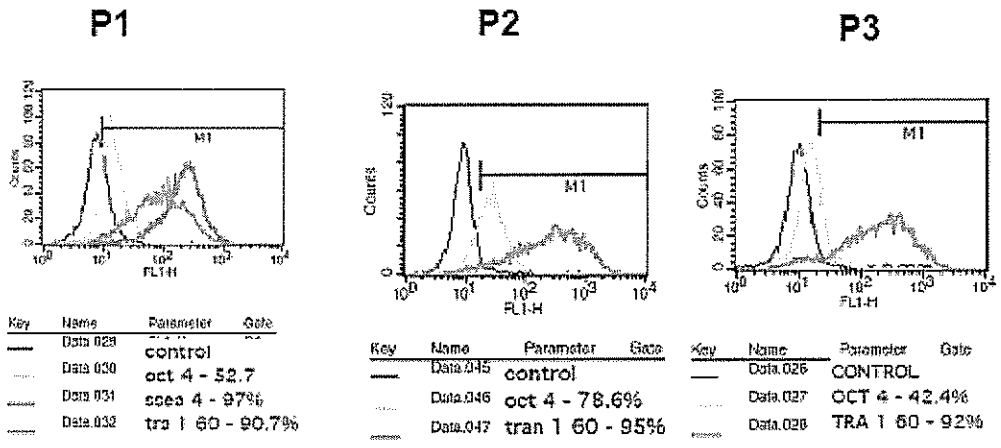


Figure 107

FACS for Cytodex 3 with matrigel with agitation at 100rpm

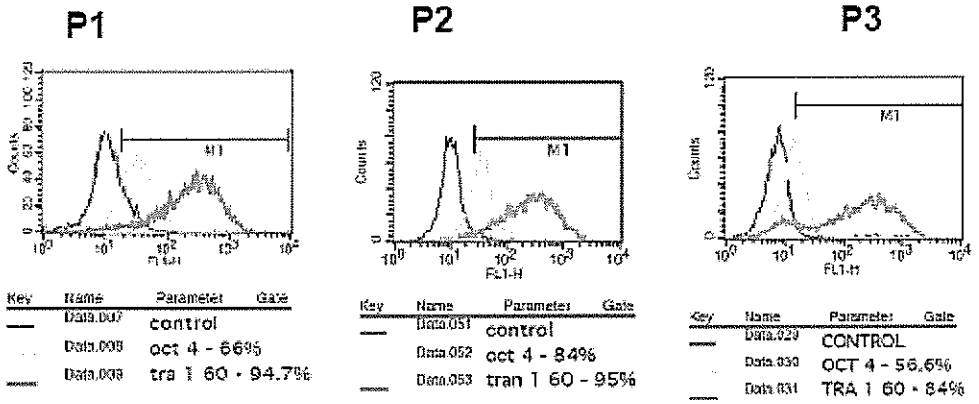


Figure 108

hESC on Cytodex 3 Microcarriers

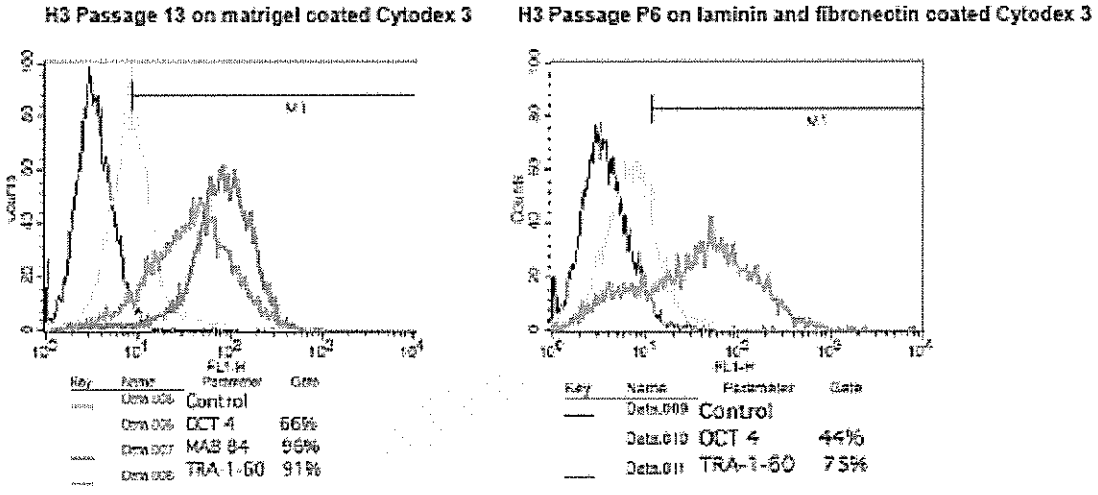


Figure 109

HES-3 P11 on Cytodex 3 with matrigel

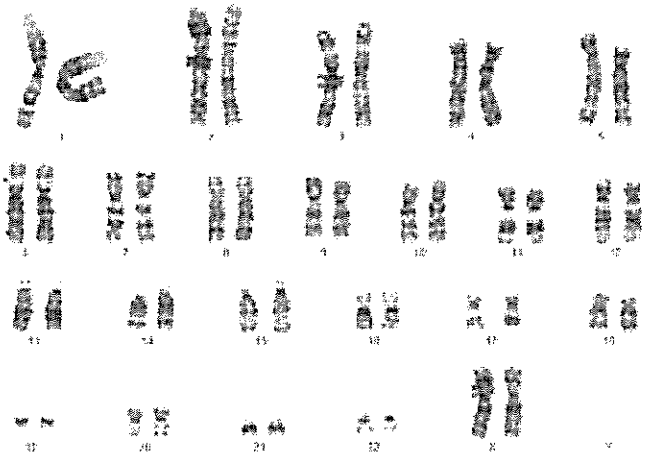
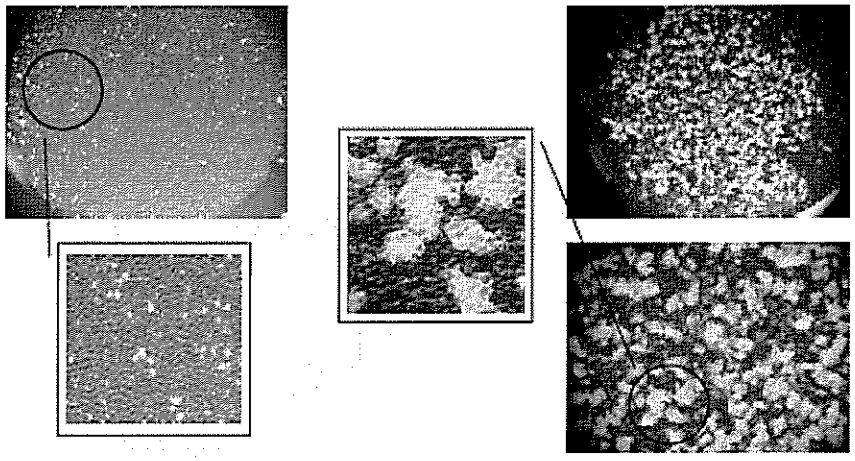


Figure 110

H3 Stem Cells passaging on Cytodex 1 microcarriers



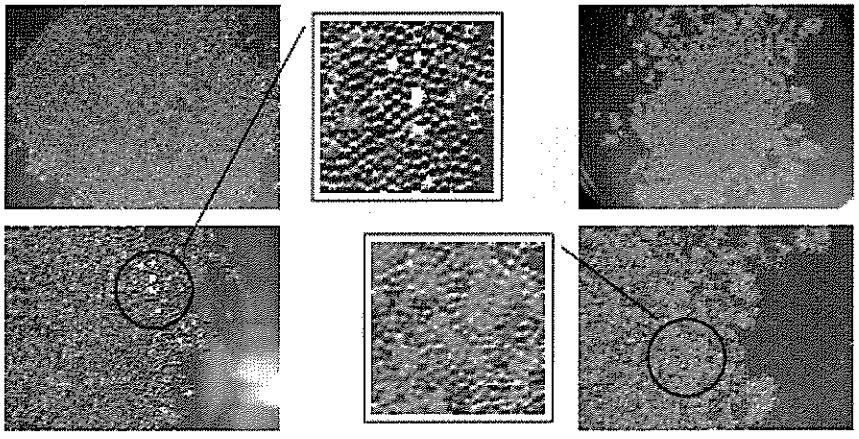
Cytodex1 (without Mgel)
Cell lightly stick on microcarriers,
Little aggregates formed.

Cytodex1 (with Mgel)
Cell stick properly on microcarriers.
Cells growth in aggregates.
Best result observed.

Figure 111

H3 Stem Cells passaging on Hillex microcarriers

HLXII adsorb phenol red. Medium becomes colorless.
Carriers with Mgel coating aggregate (even before seeding).



HLXII (without Mgel)
Cell did not stick on microcarriers,
Little clumps formed.

HLXII (with Mgel)
Lightly Cell stick observed on microcarriers,
Cells growth in aggregates

Figure 112

H3 Stem Cells passaging on microcarriers:

Summary of the cell counting (d0 and d7)

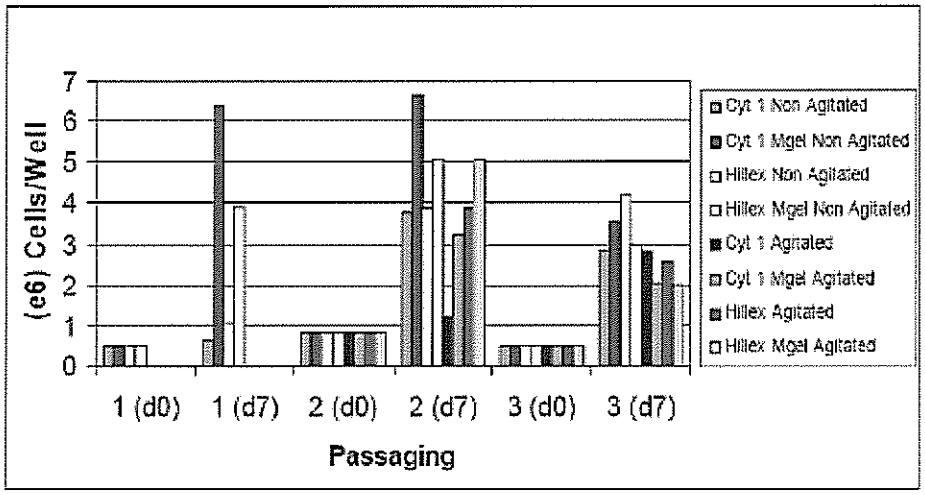


Figure 113

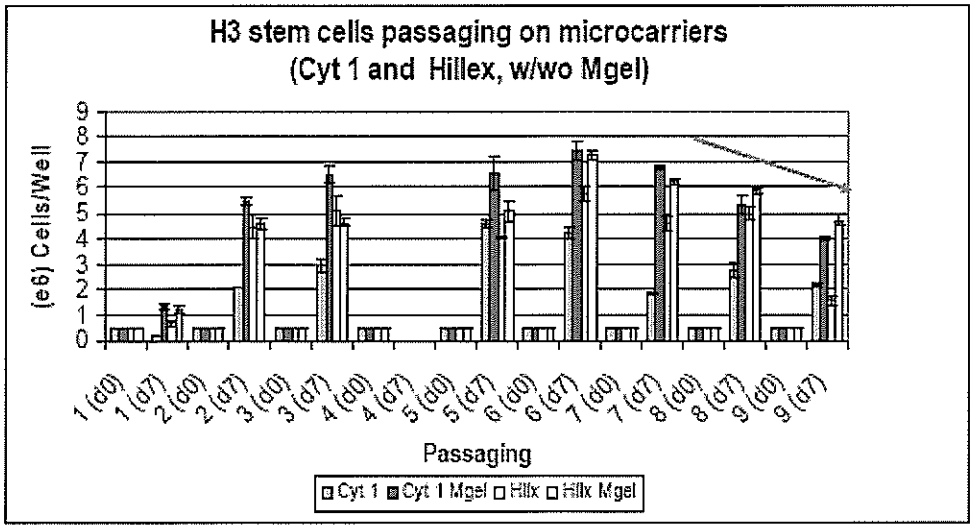
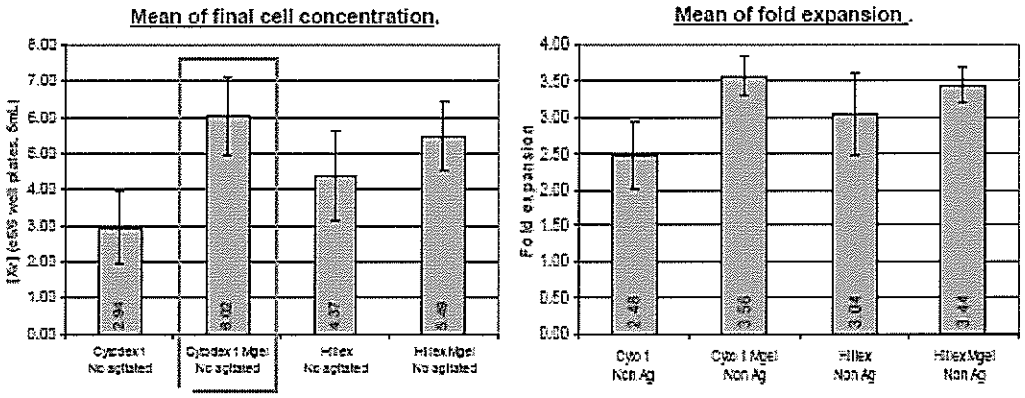


Figure 114

H3 Stem Cell passaging on microcarriers:



•Higher cell concentration has been achieved when carriers were coated with Mgel.

•Higher cell concentration has been achieved using Cytodex 1 with Mgel ($6x10^5$ cells/well or $1.2x10^6$ cells/mL), similar values as DE53 (cellulose carriers) .

Figure 115

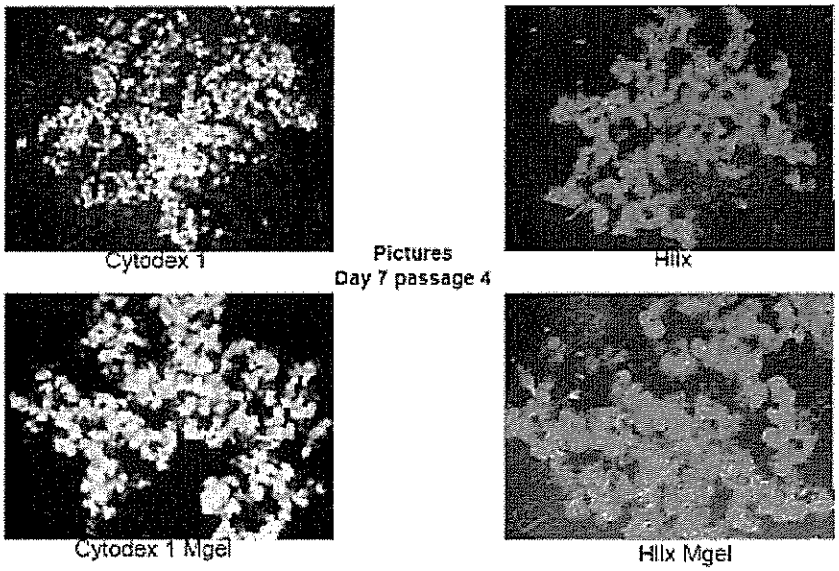


Figure 116

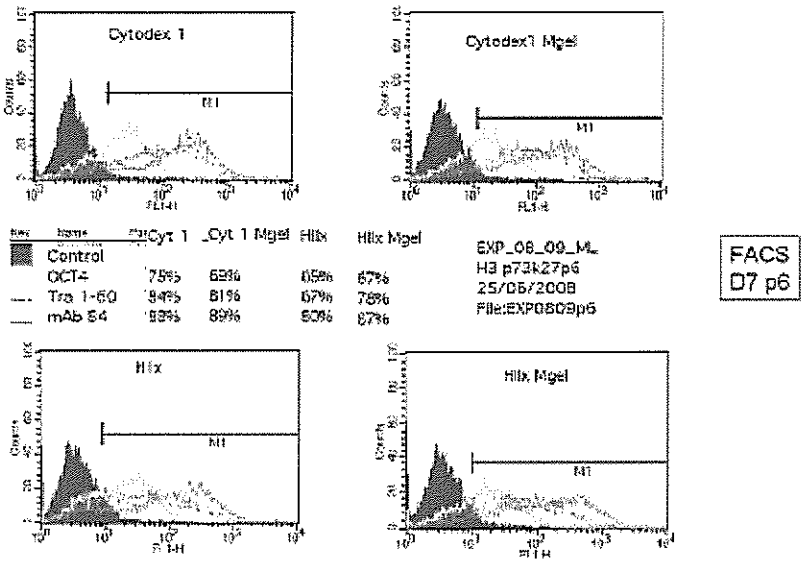
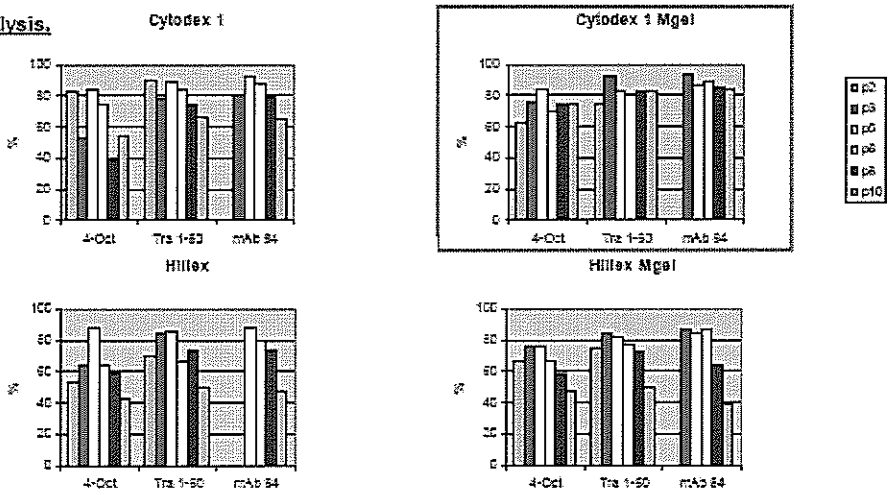


Figure 117

H3 Stem Cell passaging on microcarriers:

FACS analysis.



•Approximately after 6 passages the expression of surface markers start to decrease gradually.
•The Mgel coating provides more stability to the culture when Cytodex 1 is used, and Hillex produces similar results with and without Mgel coating.
•Cytodex 1 with Mgel is a more stable platform than Hillex; Cytodex 1 with Mgel coating is able to maintain the expression surface markers.

Figure 118

FACS at passage 13

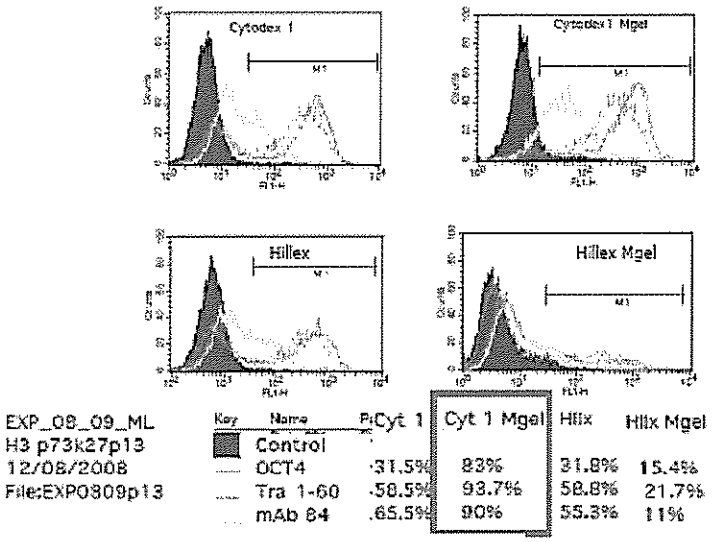


Figure 119

Karyotypes of Cytodex 1 and Hillex microcarrier cultures at passage 7

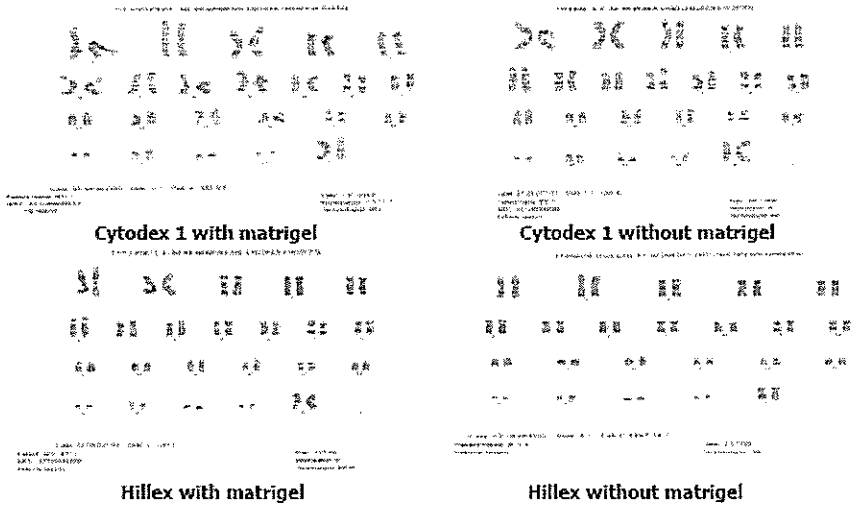


Figure 120

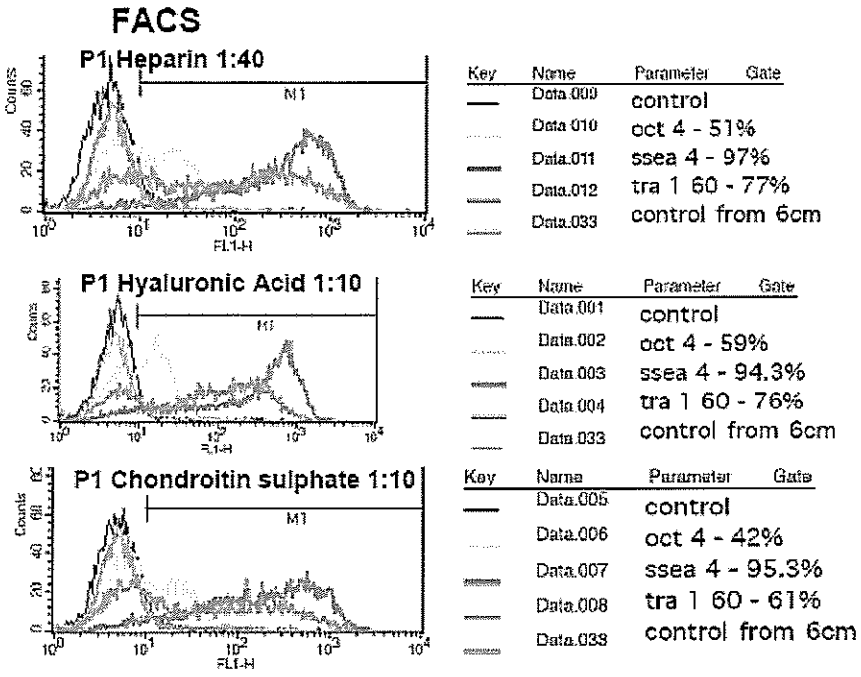


Figure 121

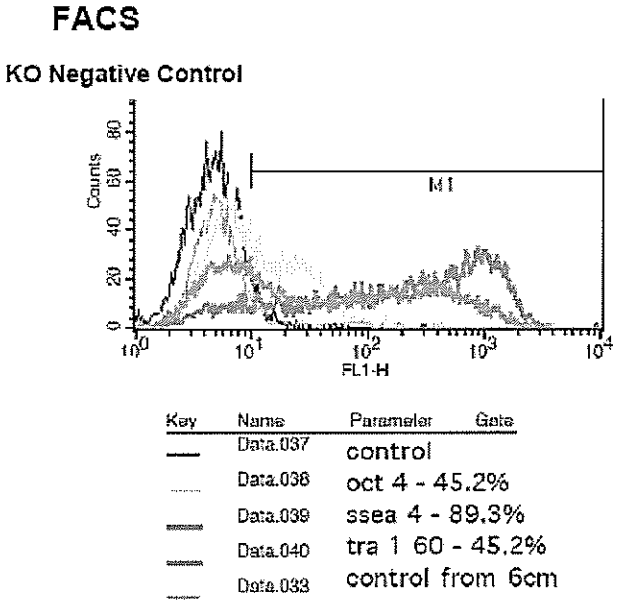
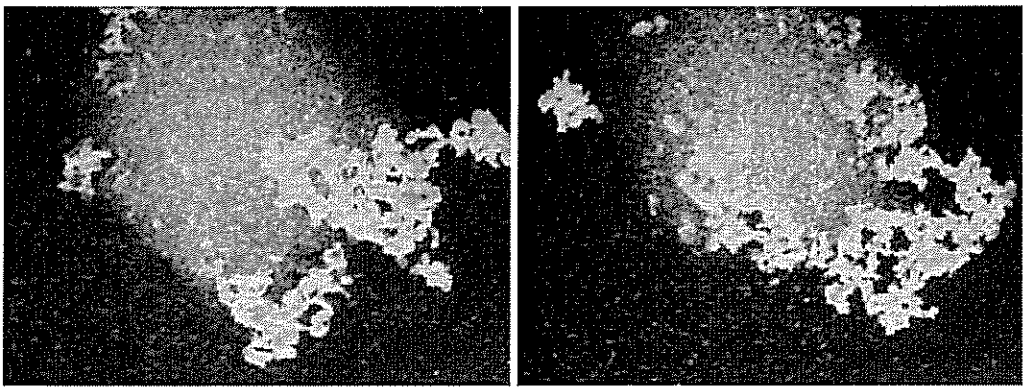


Figure 122



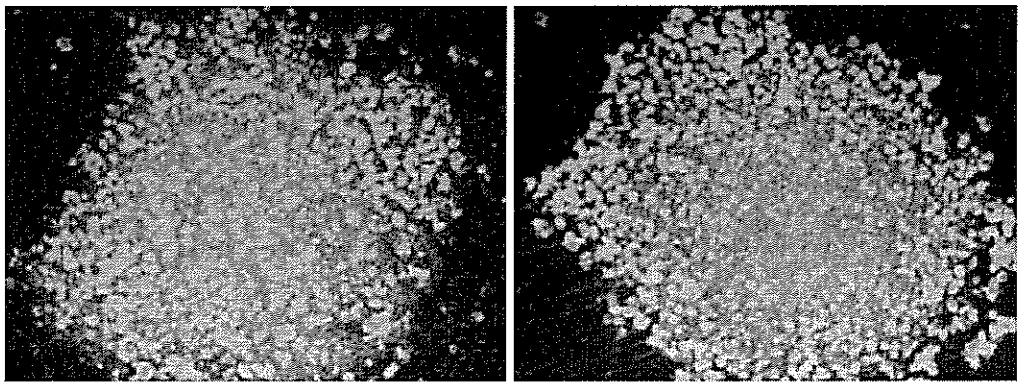
DE53 coated with HA and HS

Fibronectin (100µg) DE53 coated with HS+HA

Day 7

HA-Hyaluronic acid
HS-Heparin sodium salt

Figure 123



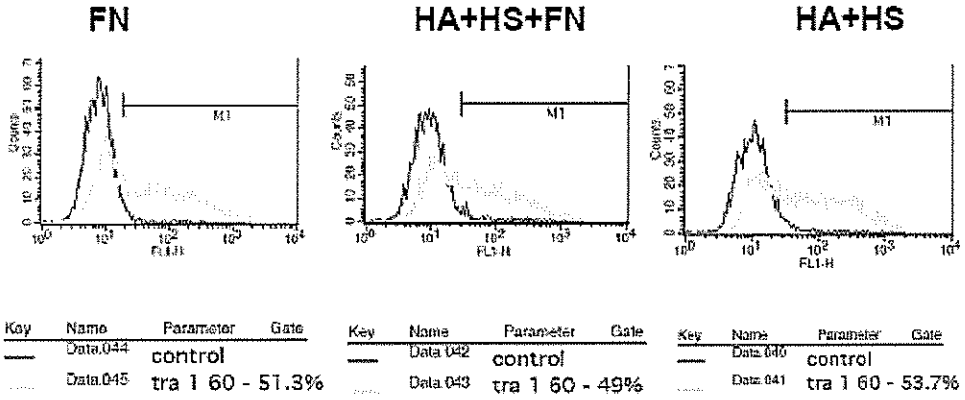
DE53 coated with HA

Fibronectin DE53 coated with HA

HA-Hyaluronic acid
HS-Heparin sodium salt

Figure 124

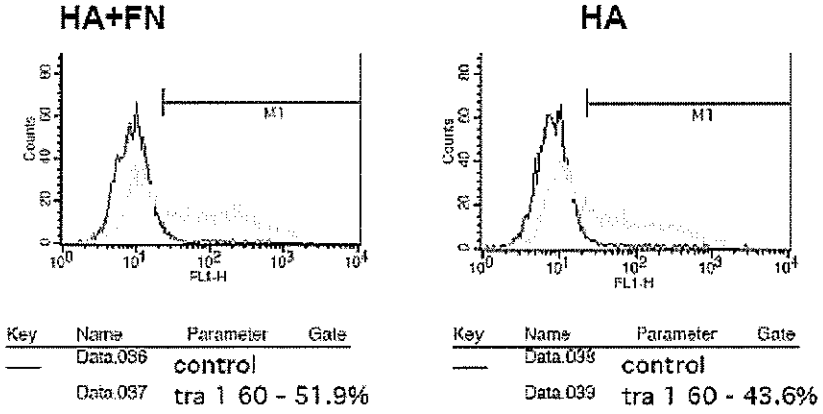
FACS for P1



FN= Fibronectin
HA= Hyaluronic Acid
HS= Heparin Sodium Salt

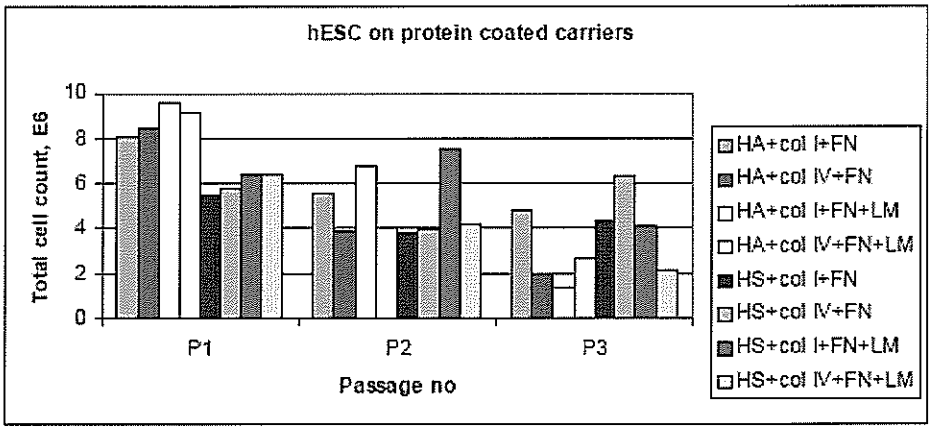
Figure 125

FACS for P1



FN= Fibronectin
HA= Hyaluronic Acid
HS= Heparin Sodium Salt

Figure 126



HA= Hyaluronic Acid **Col I= Collagen I** **FN= Fibronectin**
HS= Heparin Sodium Salt **Col IV= Collagen IV** **LM= Laminin**

Figure 127

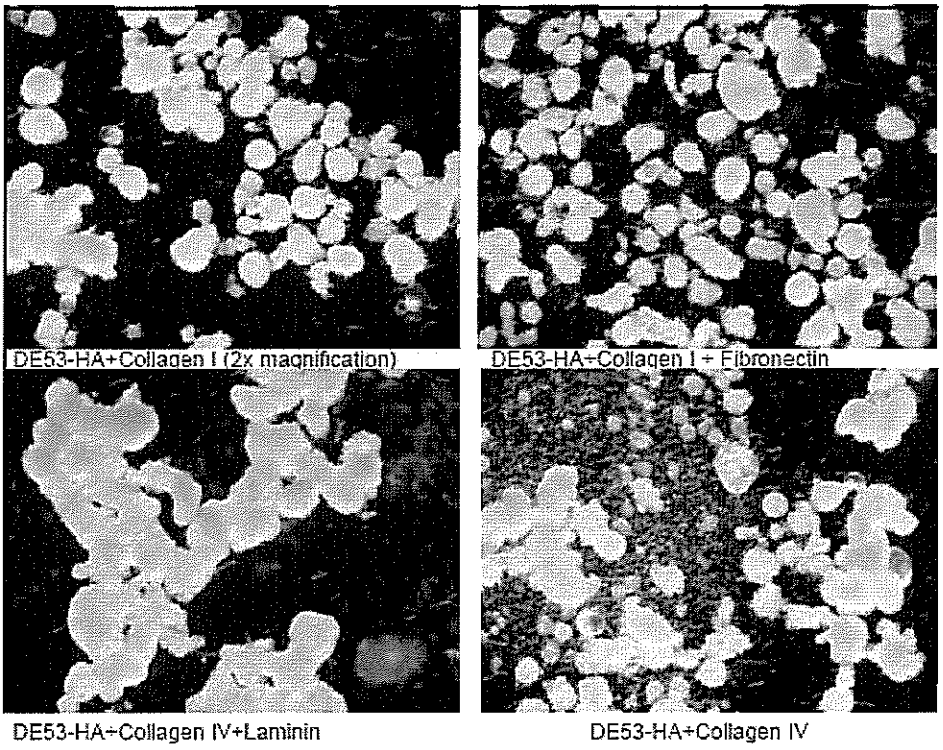


Figure 128

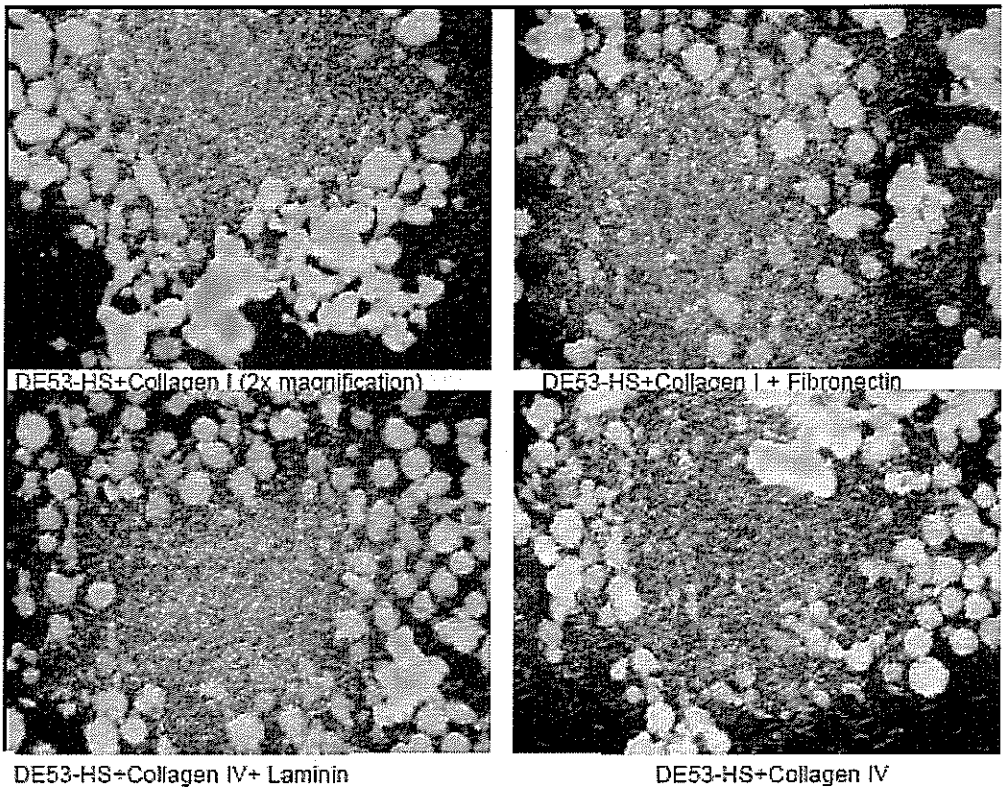


Figure 129

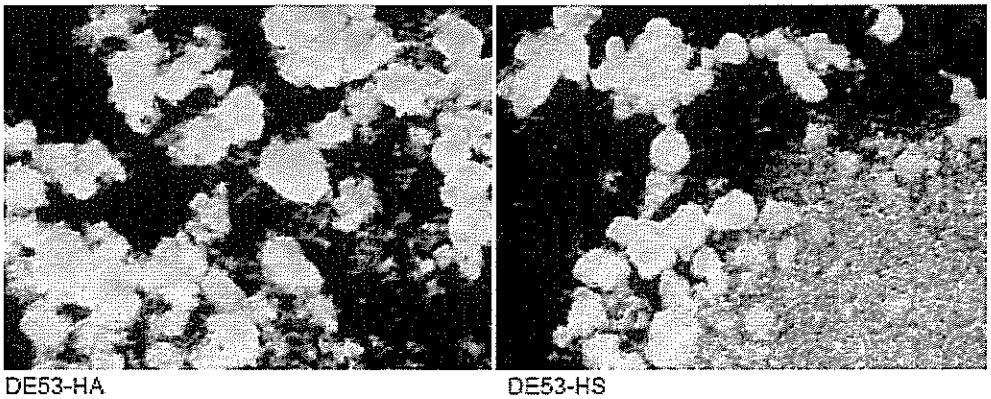


Figure 130

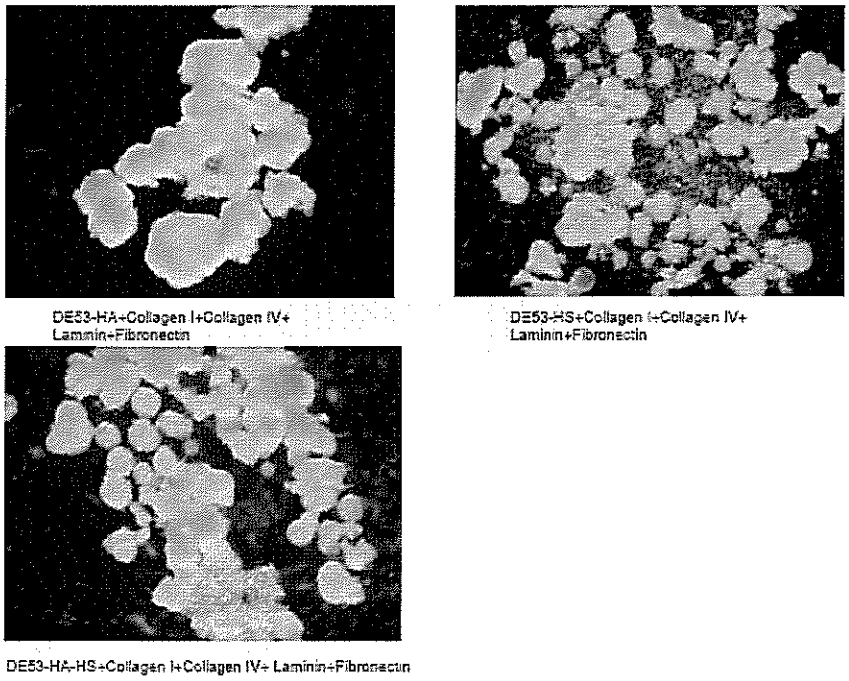
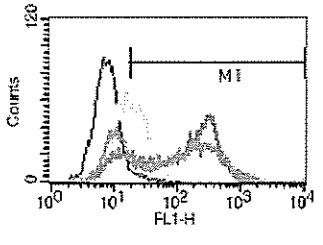


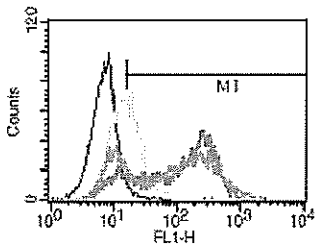
Figure 131

FACS- P3



Key	Name	Parameter	Gate
——	Data.001	control	
.....	Data.002	oct 4 - 65.7%	
———	Data.003	ssea 4- 78%	
———	Data.004	trap. l. 60 - 62.3%	

H3P33KKP59
P3 HA+COL1+FN
280308



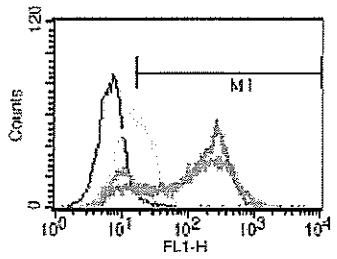
Key	Name	Parameter	Gate
——	Data.005	control	
.....	Data.006	oct 4 - 55.7%	
———	Data.007	ssea 4 - 82%	
———	Data.008	tra 1 60 - 69%	

H3P33KKP59
P3 HA+COL4+FN
280308

HA= Hyaluronic Acid **Col I= Collagen I** **FN= Fibronectin**
HS= Heparin Sodium Salt **Col IV= Collagen IV** **LM= Laminin**

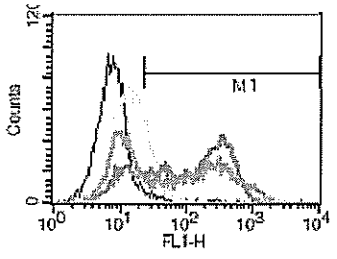
Figure 132

FACS- P3



Key	Name	Parameter	Gate
	Data 009	control	
	Data 010	oct 4 - 53.5%	
	Data 011	ssea 4- 85%	
	Data 012	tran 1 60 - 73%	

H3P33KKP59
P3 HA+COL1+FN+LM
280308



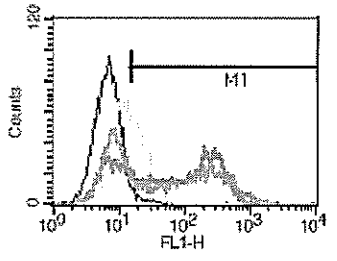
Key	Name	Parameter	Gate
	Data 013	control	
	Data 014	oct 4 - 49%	
	Data 015	ssea 4 - 74%	
	Data 016	tra 1 60 - 53%	

H3P33KKP59
P3 HA+COL4+FN+LM
280308

HA= Hyaluronic Acid **Col I= Collagen I** **FN= Fibronectin**
HS= Heparin Sodium Salt **Col IV= Collagen IV** **LM= Laminin**

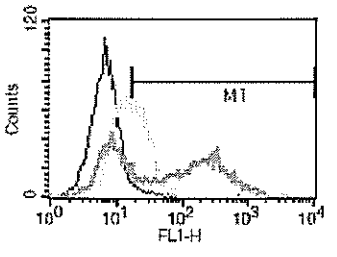
Figure 133

FACS- P3



Key	Name	Parameter	Gate
	Data.017	control	
	Data.018	oct 4 - 53%	
	Data.019	ssea 4- 65%	
	Data.020	tran 1 60 - 51%	

H3P33KKP59
P3 HS+COL1+FN
280308



Key	Name	Parameter	Gate
	Data.021	control	
	Data.022	oct 4 - 59%	
	Data.023	tra 1 60 - 57%	

H3P33KKP59
P3 HS+COL4+FN
280308

HA= Hyaluronic Acid **Col I= Collagen I** **FN= Fibronectin**
HS= Heparin Sodium Salt **Col IV= Collagen IV** **LM= Laminin**

Figure 134

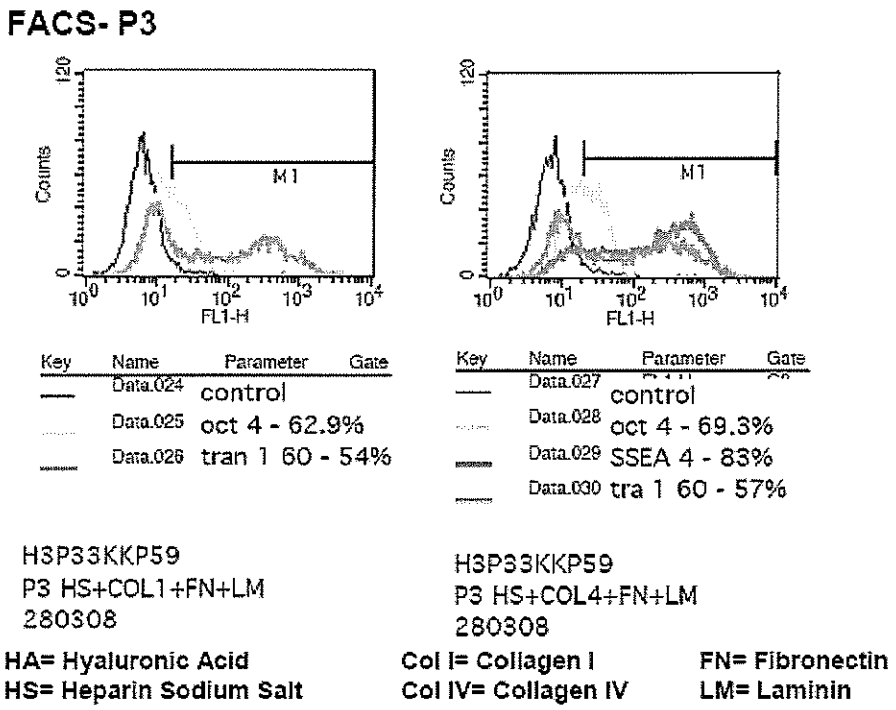


Figure 135

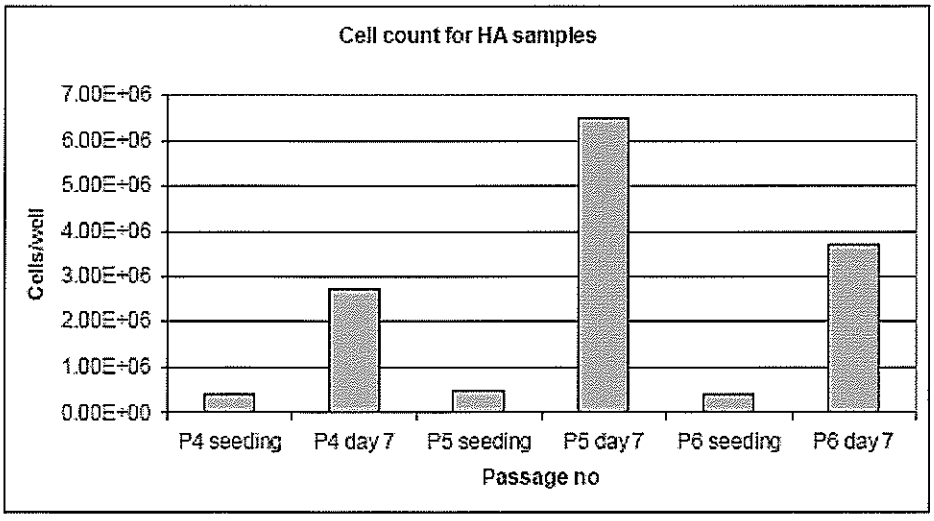


Figure 136

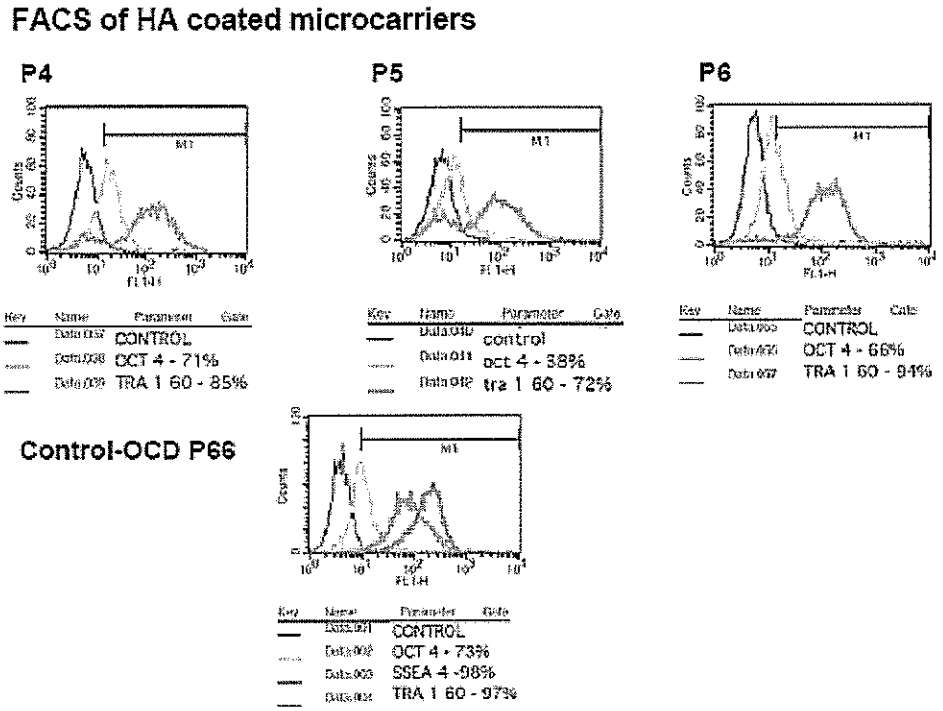


Figure 137

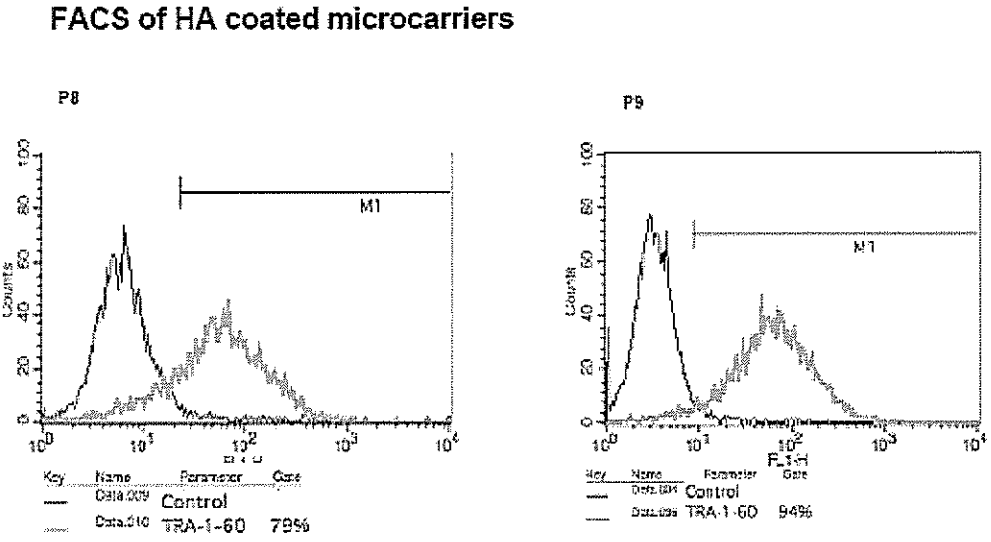


Figure 138

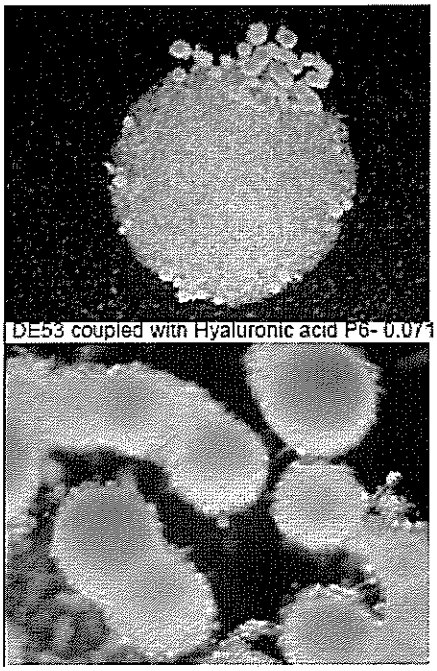


Figure 139

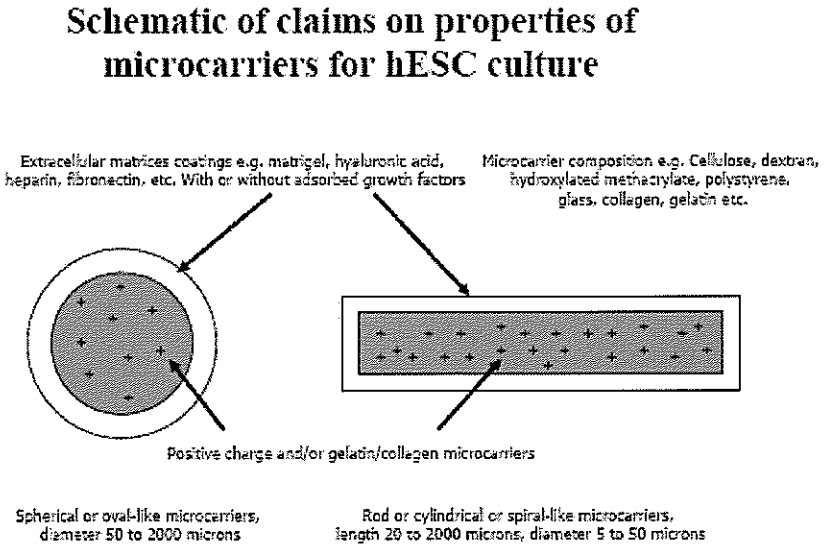


Figure 140

General Amino Acid Analysis (HPLC) of Spent Media

Table 1

Amino Acids	Consumed	No Significant Change	Production
Alanine			√
Arginine	√		
Asparagine		√	
Aspartic Acid		√	
Cystine	√		
Glutamine	√		
Glutamic Acid			√
Glycine		√	
Histidine		√	
Isoleucine	√		
Leucine	√		
Lysine		√	
Methionine	√		
Phenylalanine		√	
Proline			√
Serine	√		
Threonine		√	
Tyrosine		√	
Valine		√	

Figure 141

Amino Acid Analysis of Spent Media from StemPRO and mTeSR1

Table 2

	mTeSR1		StemPRO	
Amino Acids	Percentage(%)	Amount	Percentage(%)	Amount
Aspartic Acid	- 11.7	17→15	- 4.7	16→14
Glutamic Acid	+ 48.2	53→39	+ 31.6	38→57
Asparagine	- 4.3	23→22	-4	25→24
Serine	- 29.6	27→19	- 21.8	32→25
Histidine	0	Maintains about 22	-8	25→23
Glutamine	- 22.8	364→281	+ 23.2	191→223
Glycine	-9	25→22	0	Maintains about 27
Arginine	- 14.8	96→82	- 15.8	117→985
Threonine	0	Maintains about 47	- 5.7	53→50
Alanine	+ 18.9	32→38	- 6.9	265→241
Proline	+ 35.5	31→42	+ 20.0	30→35
Cystine	- 17.9	28→23	- 35.1	28→14
Tyrosine	0	Maintains about 34	- 7.5	40→37
Valine	-2.4	42→41	- 12.5	50→44
Methionine	- 20.0	15→12	- 23.5	17→13
Lysine	0	Maintains about 50	- 10.8	74→66
Isoleucine	0	Maintains about 39	- 17.0	47→39
Leucine	-17.8	45→37	- 36.4	47→95
Phenylalanine	0	Maintains about 30	- 11.1	36→32

- Consumption + Production

Figure 142

Co-culture of hESC with feeders on microcarriers

Table 3

	Seeding density at 8E5 cells/well	Seeding density at 0.75 to 8E5 cells/well
Passage number	P0	P1
Feeders on Cytodex 3 hESC on DE53	3.7 E6 cells/well	4.3 E6 cells/well
Feeders on Tosoh polylysine hESC on DE53	4.2 E6 cells/well	4.9 E6 cells/well
Feeders on matrigel DE53 hESC on DE 53	3.7 E6 cells/well	5.9 E6 cells/well
hESC on matrigel coated DE53 (seed at 1E6)	4.3 E6 cells/well	

Figure 143

Co-culture of hESC with feeders on microcarriers

Table 4

	Seeding density at 8E5 cells/well	Seeding density at 0.75 to 8E5 cells/well	Seeding density at 0.8 E5 to 1E6 cells/well
Passage number	P0	P1	P2
Feeders on cytodex 3 hESC on DE53	3.7 E6 cells/well	4.3 E6 cells/well	4.05 E6 cells/well
Feeders on tosoh polylysine hESC on DE53	4.2 E6 cells/well	4.9 E6 cells/well	3.4 E6 cells/well
Feeders on matrigel DE53 hESC on DE 53	3.7 E6 cells/well	5.9 E6 cells/well	4.7 E6 cells/well
hESC on matrigel coated DE53 (seed at 1E6)	4.3 E6 cells/well	2 E6 cells/well	2 E6 cells/well

Figure 144

hESC culture on Tosoh carriers

Table 5

Type of bead	Particle size	Coating	Passage 0	Passage 1
Toyopearl TSKgel Tresyl 5Pw	10µm	4mg protamine (96 mg protamine/g dry beads)	2 E6 cells/well	4.4e5 cells/well
Toyopearl TSKgel Tresyl 5Pw	10µm	0.2mg protamine (4.8mg protamine/g dry beads) + mgel	1.99 E6 cells/well	5.98 cells/well
Toyopearl TSKgel Tresyl 5Pw	10µm	4mg protamine + mgel	1.64 E6 cells/well	4.56 cells/well
Toyopearl AF-Tresyl-650M	65µm	4mg protamine + mgel	1.71 E6 cells/well	6.18 cells/well

Total nuclei count on day 7.
Note: Seeding density at 1E6 cells/well (5ml media per well) for passage 0 and split ratio of 1:4 for passage 1

Figure 145

Cells were seeded at 4 to 8 E5 cells/well and counts taken at day 7

Nuclei count results

Table 6

Large 65 micron Tosoh microcarriers (Sample ID)	Passage 0, E6 Cells/ml	Passage 1, E6 Cells/well	Passage 2, E6 Cells/well	Passage 3, E6 Cells/well	Passage 4, E6 Cells/well
Poly-lysine (PL)	4.74	3.37	1.3	1.73	NA
Polylysine coupled with matrigel (PLMG)	3.18	3.29	2.7	2.46	NA
Poly lysine coated with matrigel (PL+MG)	3.72	3.40	6.2	3.2	4.36
Protamine (PR)	3.9	4.30	1.2	1.99	NA
Protamine coupled with matrigel (PRMG)	4.32	3.80	1.5	5.55	NA
Protamine coated with matrigel (PR+MG)	4.5	3.46	3.2	5.15	5

Figure 146

hESC on Cytodex 3 microcarriers

Nuclei count results

Table 7

Sample ID	P0, E6 cells	P1, E6 cells	P3, E6 cells
Static- cytodex 3 w/o matrigel	0.5	2	5.2
Static- cytodex 3 with matrigel	1.7	9.9	8.1
Agitation- cytodex 3 w/o matrigel	1.2	1.2	4.6
Agitation- cytodex 3 with matrigel	1.67	3.9	6.9

Note: Seeding density at 8E5 cells/well and counts taken at day 7

Figure 147

P0 of new extracellular matrix coatings on DE53 cellulose carriers

Cells were seeded at 4 E5 cells/well and counts taken at day 7
Control: KO = 4.3 E5 cells/well; CM = 4.4 E5 cells/well

Table 8

Dilution ratio	Chondroitin Sulfate (Stock conc. 0.41 g/ml)	Heparin (Stock conc. 0.2 g/ml)	Hyaluronic Acid (Stock conc. 7.09 mg/ml)
1:10	NA	9.6 E5 cells/well	1.17 E6 cells/well
1:20	8.3 E5 cells/well	1.03 E6 cells/well	7.7 E5 cells/well
1:40	6.5 E5 cells/well	1.18 E6 cells/well	8.3 E5 cells/well
1:80	5.5 E5 cells/well	1.12 E6 cells/well	5.4 E5 cells/well

Figure 148

P1 of new extracellular matrix coatings on DE53 cellulose carriers

Cells were seeded at 4 E5 cells/well and counts taken at day 7
Control: KO = 1.42 E6 cells/well

Table 9

Dilution ratio	Chondroitin Sulfate (Stock conc. 0.41 g/ml)	Heparin (Stock conc. 0.2 g/ml)	Hyaluronic Acid (Stock conc. 7.09 mg/ml)
1:10	1.53 E6 cells/well	1.58 E6 cells/well	1.67 E6 cells/well
1:20	1.84 E6 cells/well	1.26 E6 cells/well	1.33 E6 cells/well
1:40	2.09 E6 cells/well	1.47 E6 cells/well	1.44 E6 cells/well
1:80	1.72 E6 cells/well	1.58 E6 cells/well	1.17 E6 cells/well

Figure 149

P1 of new extracellular matrix coatings on DE53 cellulose carriers

Table 10

Sample ID	Passage 0, E6 Cells/well	Passage 1, E6 Cells/well
Fibronectin (FN)	8.2	2.98
Hyaluronic Acid (HA), Heparin Sodium Salt (HS), FN	3.9	0.972
HA, HS	3.0	1.42
HA, FN	9.0	5.18
HA	10.2	1.11

Cells were seeded at 4 E5 cells/well and counts taken at day 7

Figure 150

P1 and P2 of protein coatings (with collagen I, IV and Laminin)

Cells were seeded at 4 E5 cells/well and counts taken at day 7

Nuclei count results Table 11

Sample ID	P1, E6 cells	P2, E6 cells	P3, E6 cells
HA+col I+FN	8.07	5.59	4.82
HA+col IV+FN	8.46	3.87	2.00
HA+col I+FN+LM	9.56	6.78	1.37
HA+col IV+FN+LM	9.12	4.02	2.61
HS+col I+FN	5.49	3.78	4.35
HS+col IV+FN	5.82	3.9	6.26
HS+col I+FN+LM	6.36	7.53	4.16
HS+col IV+FN+LM	6.39	4.2	2.14

HA= Hyaluronic Acid **Col I= Collagen I** **FN= Fibronectin**
HS= Heparin Sodium Salt **Col IV= Collagen IV** **LM= Laminin**

Figure 151

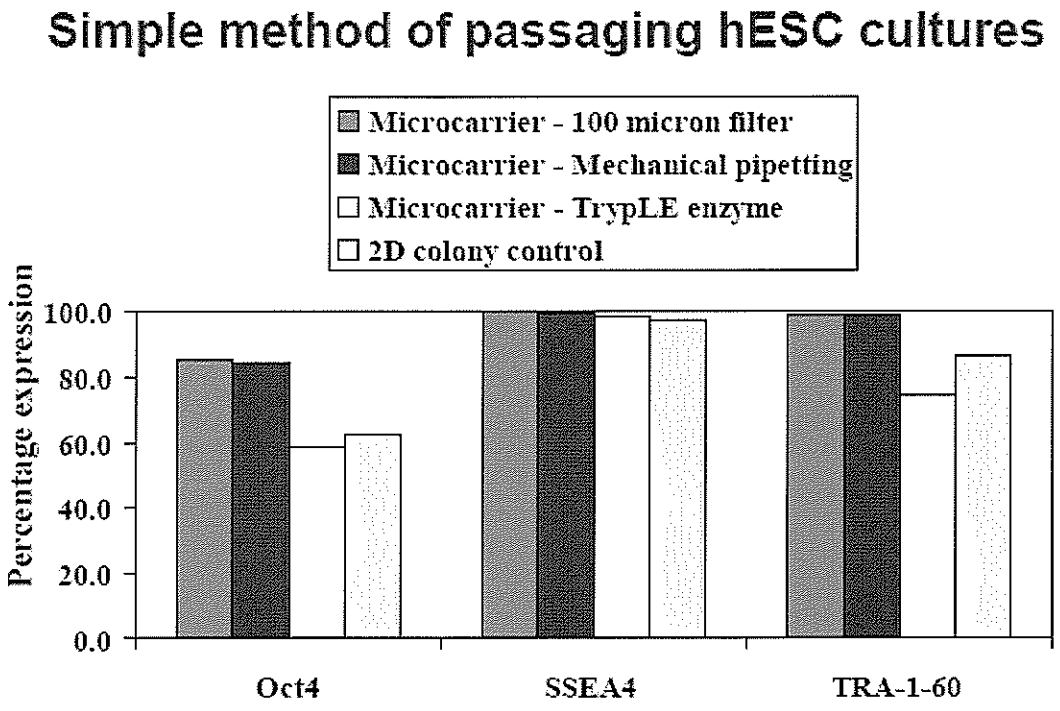

Simple method of passaging hESC cultures
Microcarrier - 100 micron filter
Microcarrier - Mechanical pipetting
Microcarrier - TrypLE enzyme
2D colony control
Percentage expression
100.0
80.0
60.0
40.0
20.0
0.0
Oct4
SSEA4
TRA-1-60

Figure 152

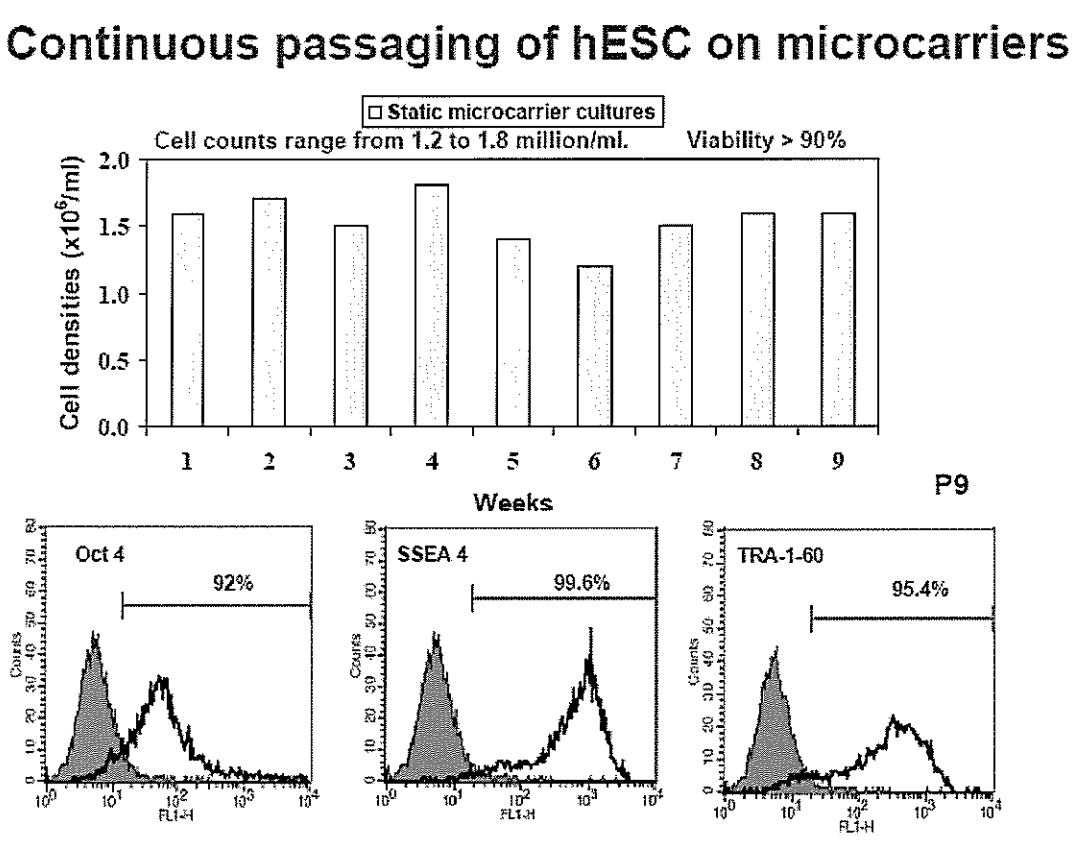

Continuous passaging of hESC on microcarriers
Static microcarrier cultures
Cell counts range from 1.2 to 1.8 million/ml.
Viability > 90%
Cell densities (x10^6/ml)
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
7
8
9
Weeks
P9
Oct 4
92%
SSEA 4
99.6%
TRA-1-60
95.4%
Counts
FL1-H

Figure 153

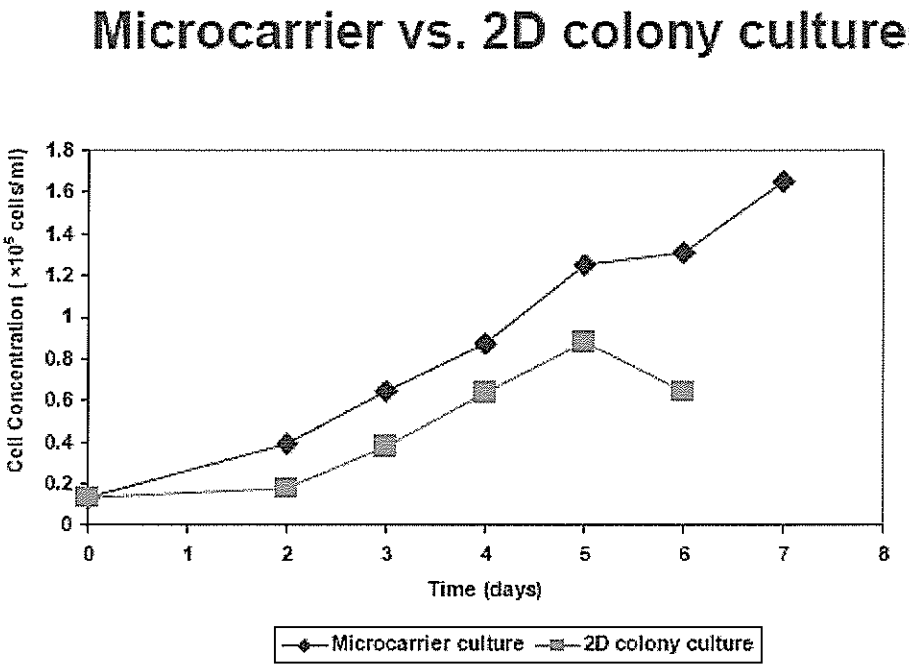
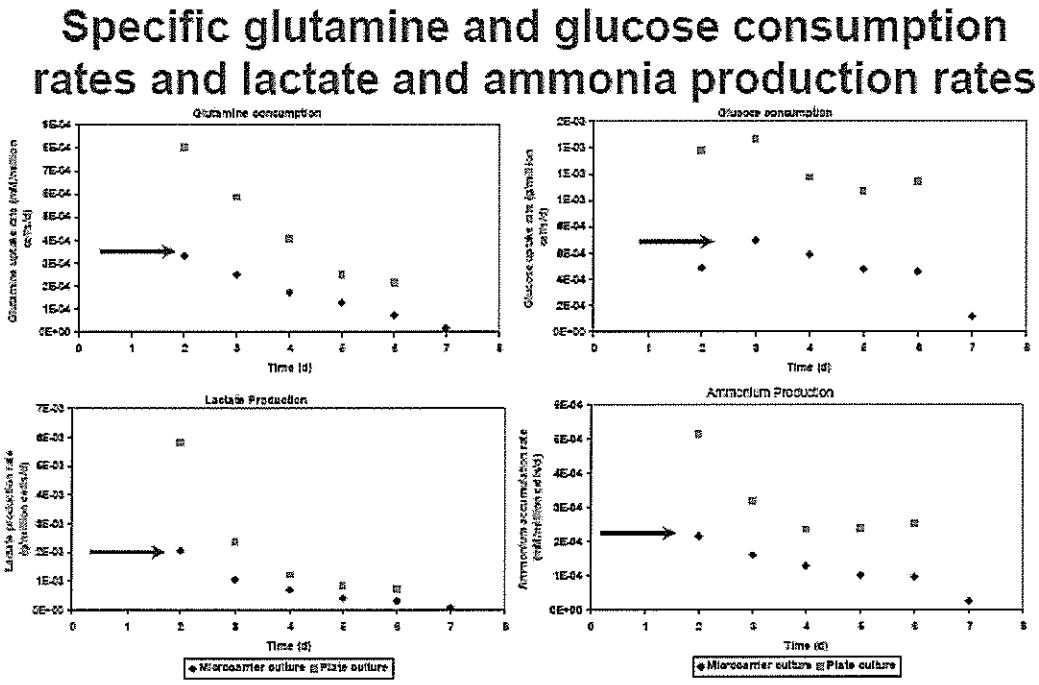

Microcarrier vs. 2D colony cultures
Cell Concentration (×10^5 cells/ml)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
6
7
8
Time (days)
Microcarrier culture
2D colony culture

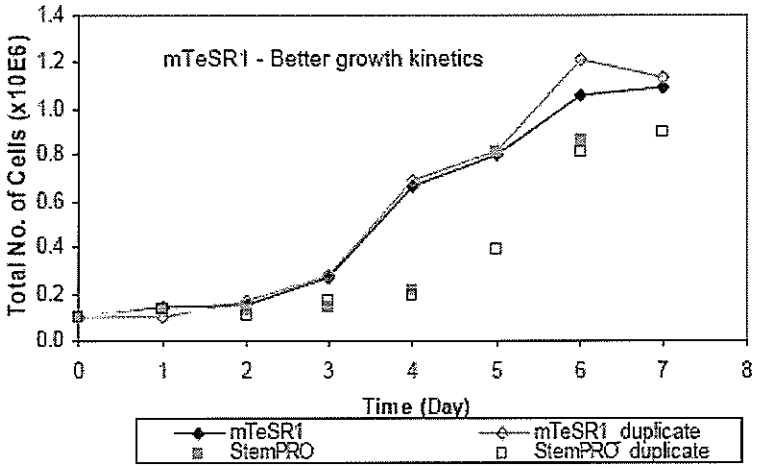
Figure 154



More efficient metabolism on microcarriers vs. 2D colony cultures

Figure 155

Microcarriers cultures in defined media



	mTeSR1	StemPRO
Growth Rate, μ (h^{-1})	0.0303	0.0197
Doubling Time (h)	23	35

Figure 156

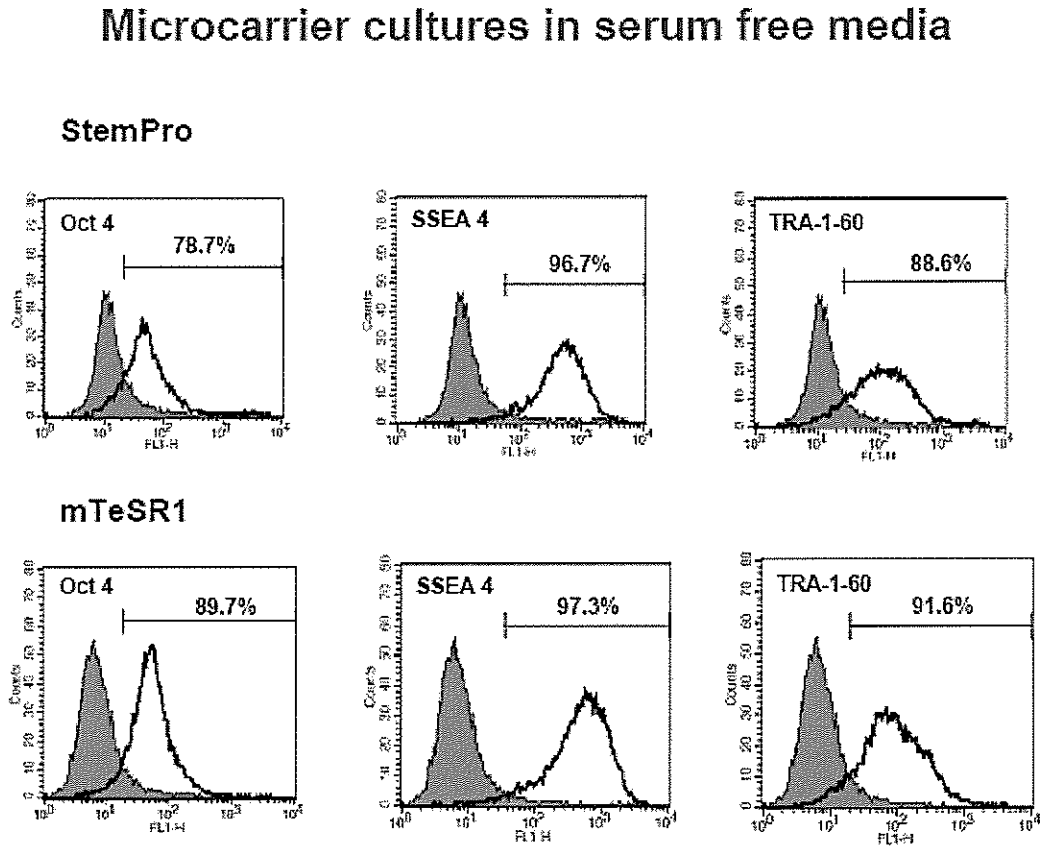

Microcarrier cultures in serum free media
StemPro
Oct 4
78.7%
SSEA 4
96.7%
TRA-1-60
88.6%
mTeSR1
Oct 4
89.7%
SSEA 4
97.3%
TRA-1-60
91.6%

Figure 157

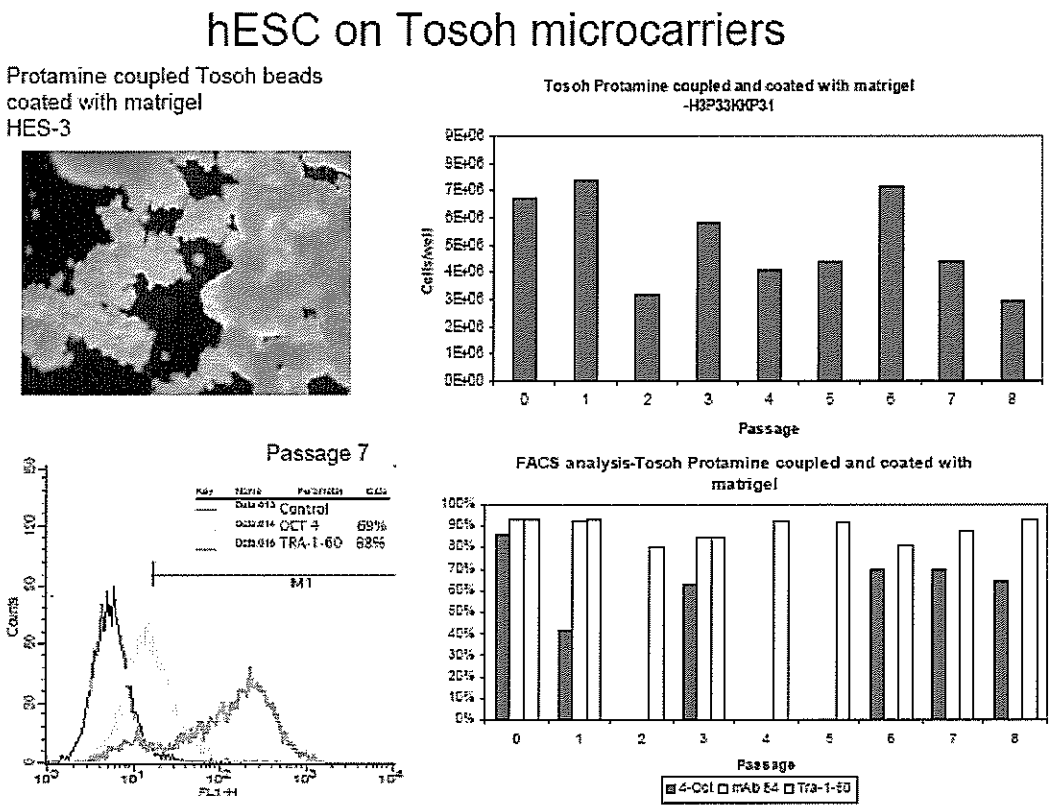

hESC on Tosoh microcarriers
Protamine coupled Tosoh beads
coated with matrigel
HES-3
Tosoh Protamine coupled and coated with matrigel
-H3P33KKP31
Cells/well
Passage
Passage 7
Control
OCT-4 69%
TRA-1-60 88%
M1
Counts
FACS analysis-Tosoh Protamine coupled and coated with
matrigel
Passage
4-Col mAb 84 Tra-1-60

Figure 158

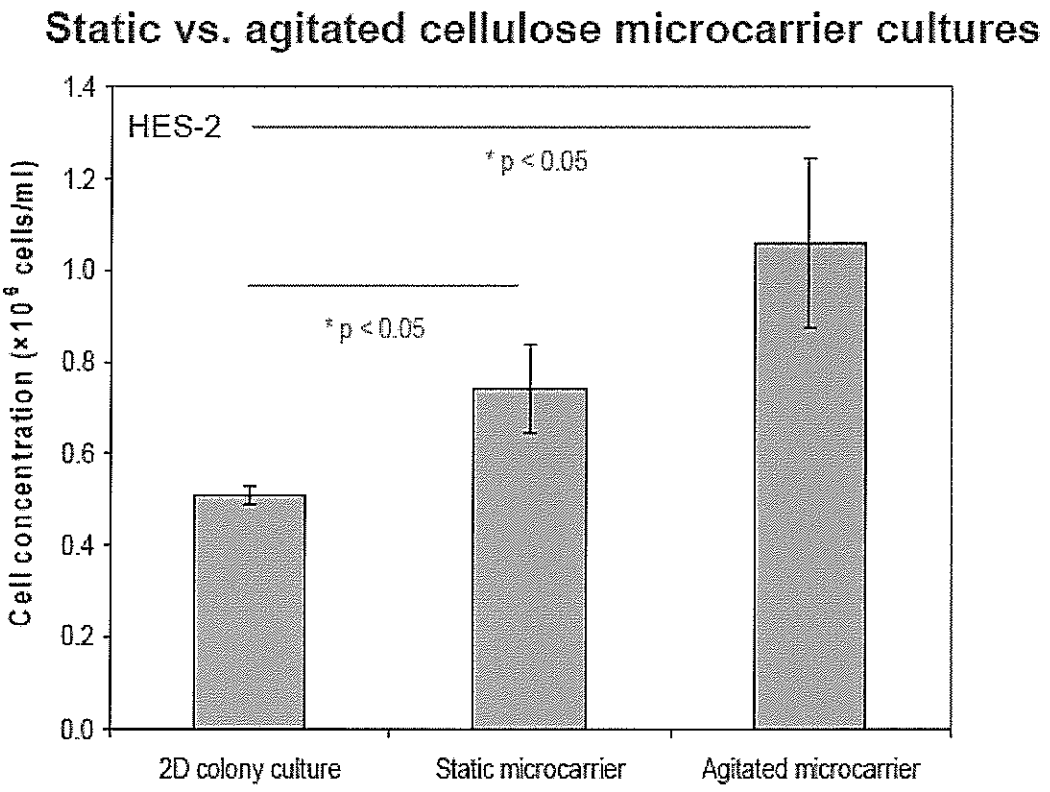

Static vs. agitated cellulose microcarrier cultures
HES-2
* p < 0.05
* p < 0.05
Cell concentration (×10 6 cells/ml)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
2D colony culture
Static microcarrier
Agitated microcarrier

Figure 159

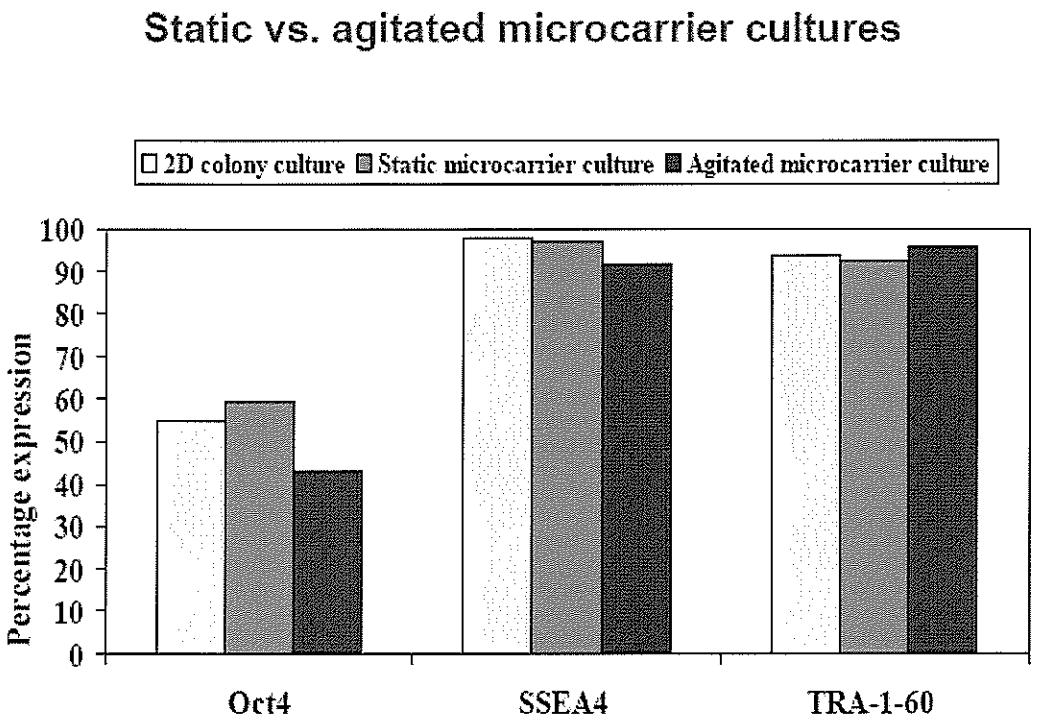

Static vs. agitated microcarrier cultures
2D colony culture
Static microcarrier culture
Agitated microcarrier culture
Percentage expression
100
90
80
70
60
50
40
30
20
10
0
Oct4
SSEA4
TRA-1-60

Figure 160

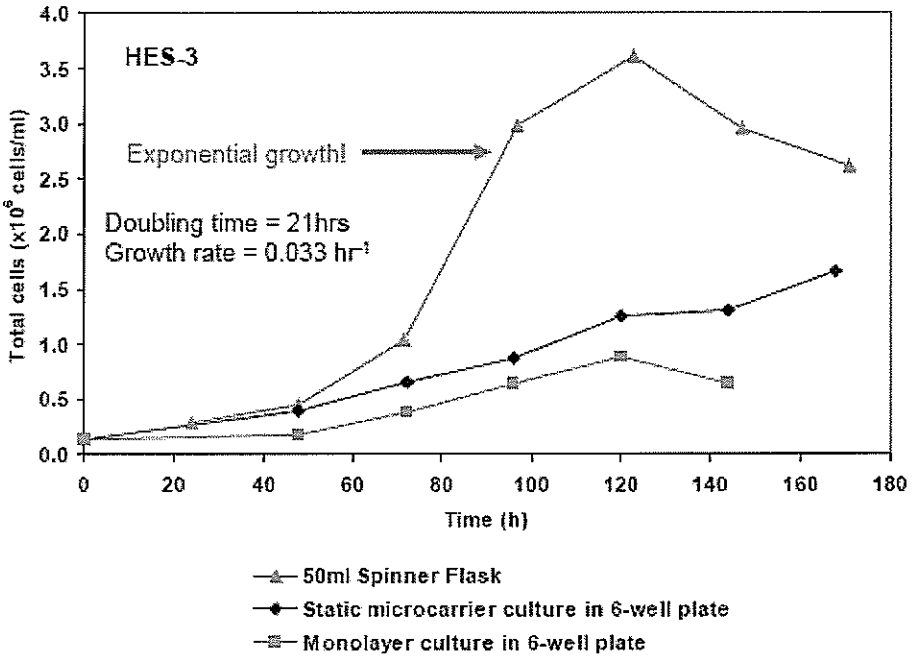

Microcarriers culture in 50 ml spinner flask
HES-3
Exponential growth!
Doubling time = 21hrs
Growth rate = 0.033 hr-1
Total cells (x10^6 cells/ml)
Time (h)
50ml Spinner Flask
Static microcarrier culture in 6-well plate
Monolayer culture in 6-well plate

Figure 161

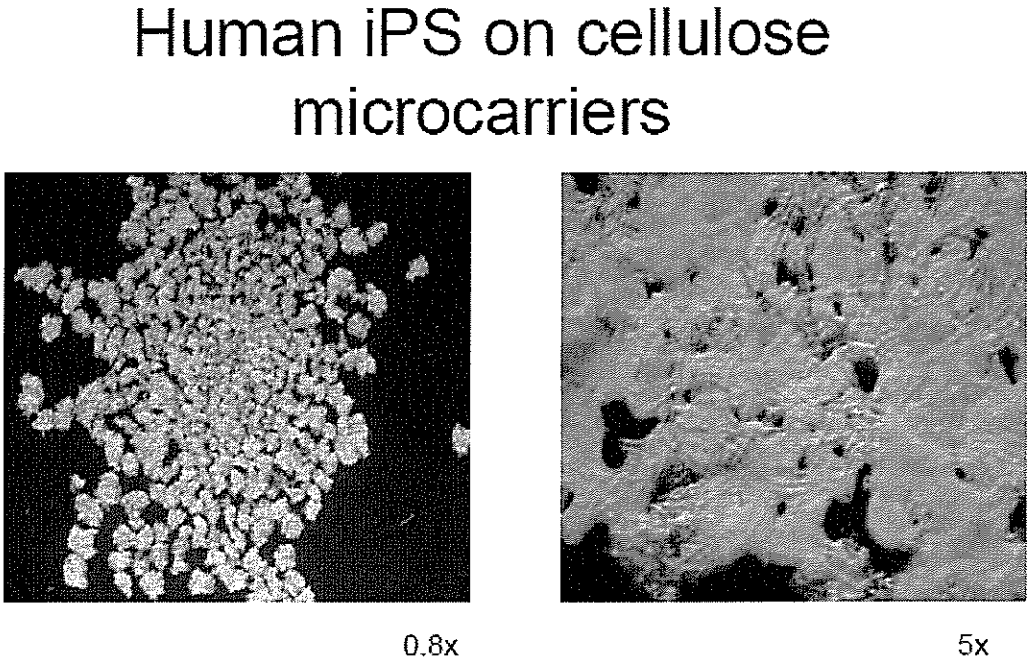


Figure 162

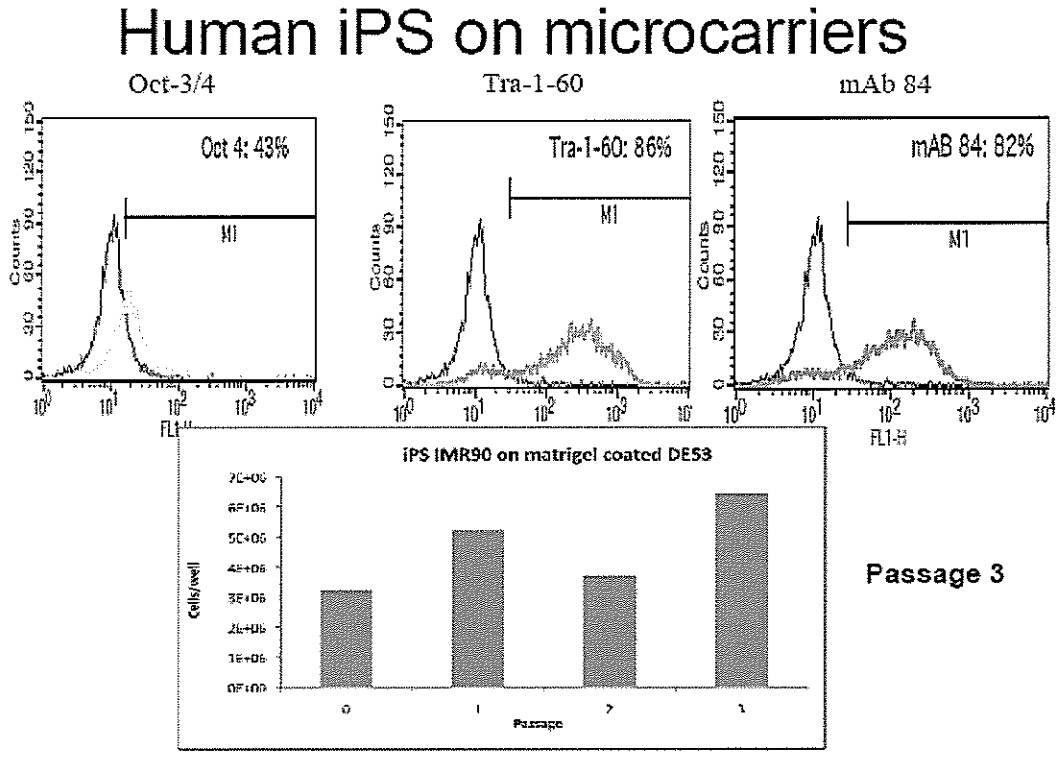
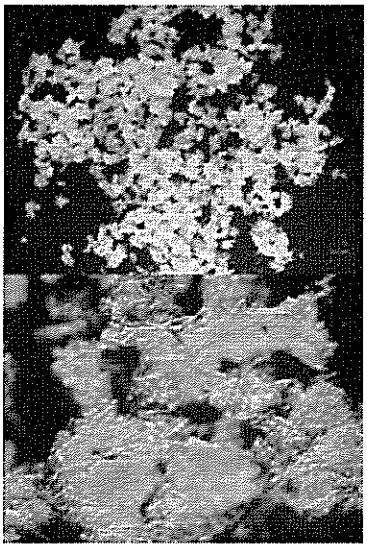

Human iPS on microcarriers
Oct-3/4
Tra-1-60
mAb 84
Oct 4: 43%
Tra-1-60: 86%
mAB 84: 82%
M1
Counts
FL1-H
iPS IMR90 on matrigel coated DE53
Cells/well
Passage
Passage 3

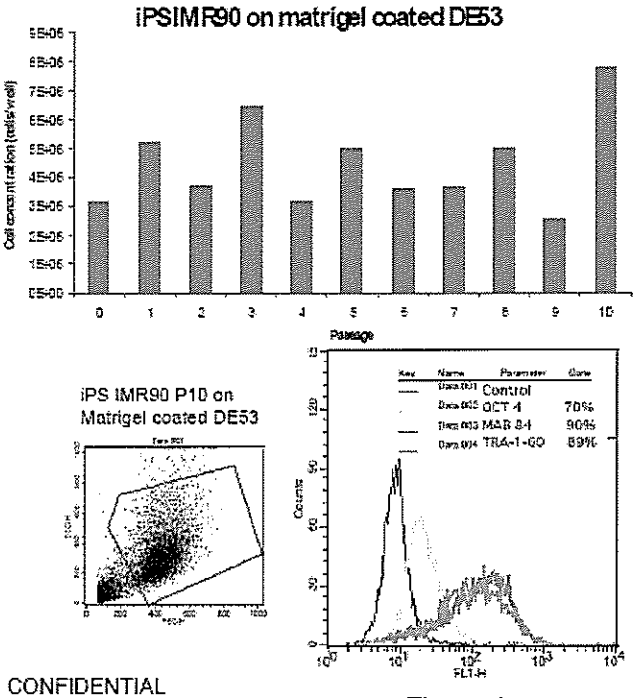
Figure 163

Human iPS Cells (IMR90)

Matrigel coated DE53 microcarrier- 20 mg/well (4mg/ml)
MEF conditioned KO-medium with 100ng/ml bFGF (5ml/well)

Seeded from iPS IMR90P8MGP5
(8 passages of cultivation on feeders followed by adaptation on matrigel for 5 passages)





CONFIDENTIAL

Figure 1

Figure 164

Microcarriers	Coatings
DE53 cellulose (3 mg/ml)	Conditioned media (uncoated)
DE53 cellulose (4 mg/ml)	Laminin (4 ug/ml)
DE53 cellulose (4 mg/ml)	Fibronectin (20 ug/ml)
DE53 cellulose (4 mg/ml)	Vitronectin (4 ug/ml)
Tosoh 65 protamine (9,600 beads/ml)	Conditioned media (uncoated)
Tosoh 65 protamine (9,600 beads/ml)	Laminin (4 ug/ml)

Figure 165

Differentiation on cellulose microcarriers with different coatings

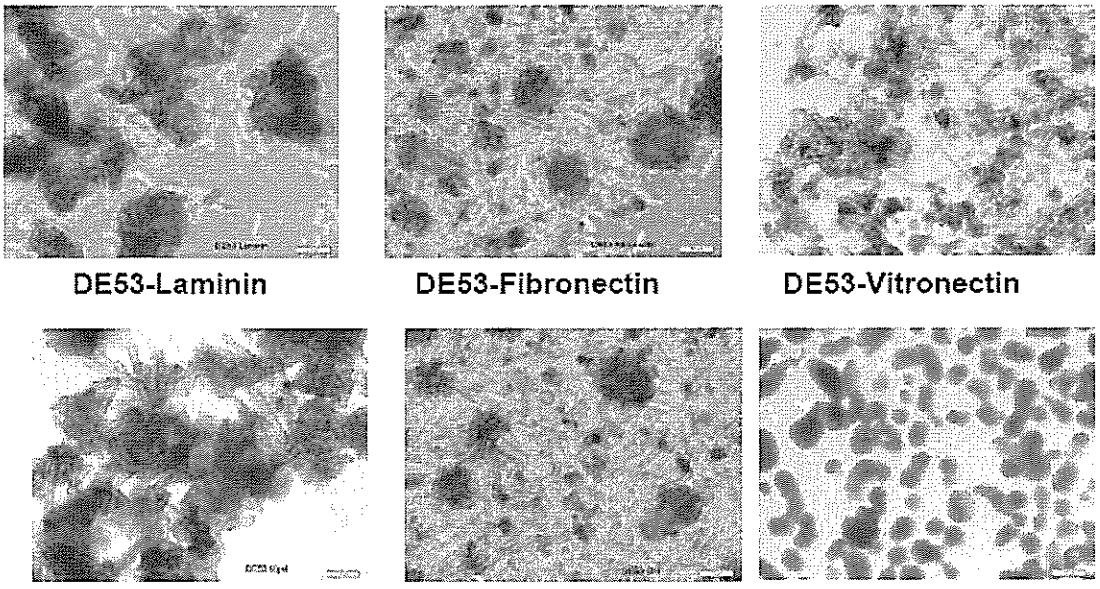


Figure 166

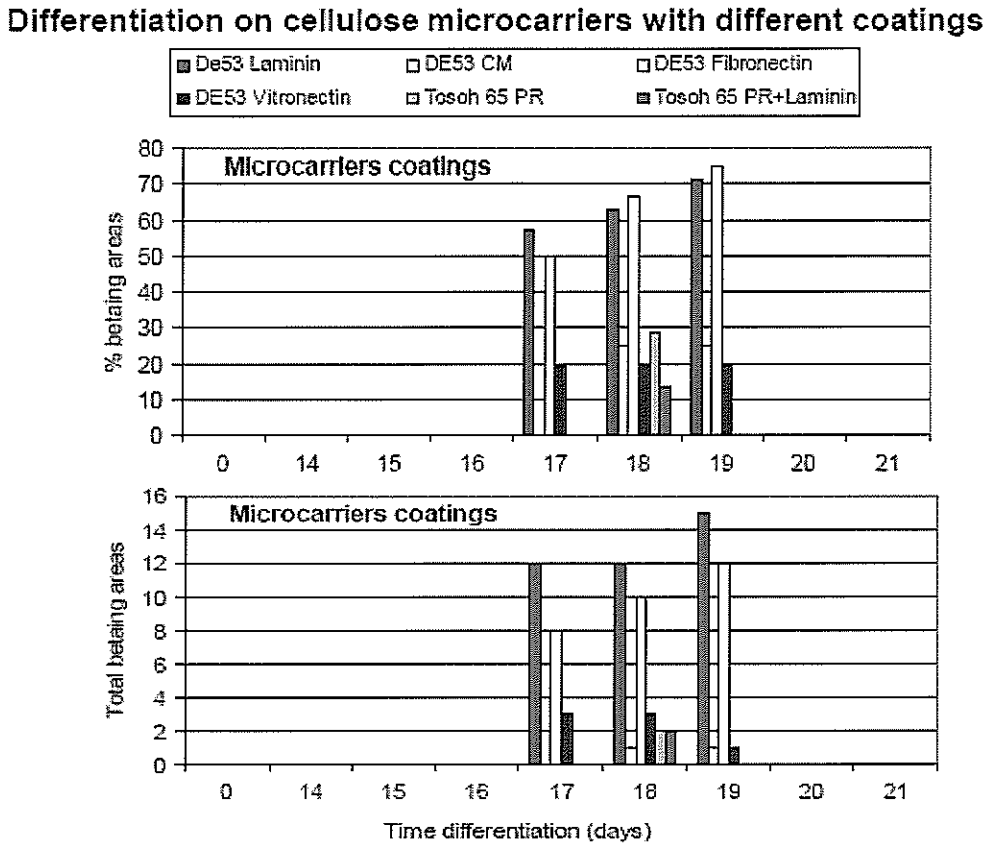

Differentiation on cellulose microcarriers with different coatings
De53 Laminin
DE53 CM
DE53 Fibronectin
DE53 Vitronectin
Tosoh 65 PR
Tosoh 65 PR+Laminin
Microcarriers coatings
% betaing areas
Microcarriers coatings
Total betaing areas
Time differentiation (days)

Figure 167

Expansion and differentiation with different ECM coatings

Day 14 aggregates

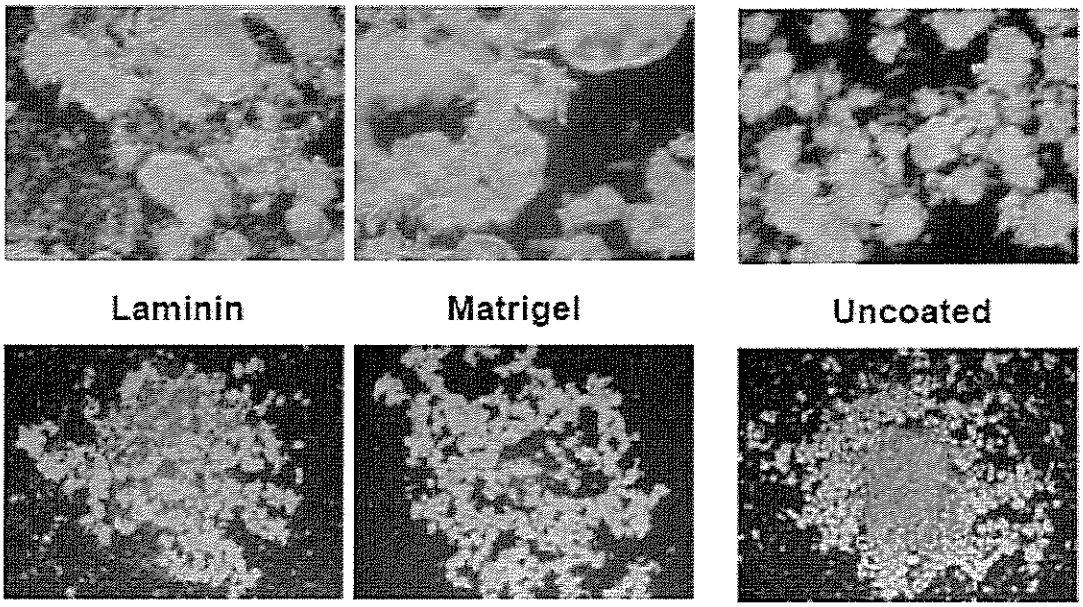


Figure 168

Expansion and differentiation with different ECM coatings

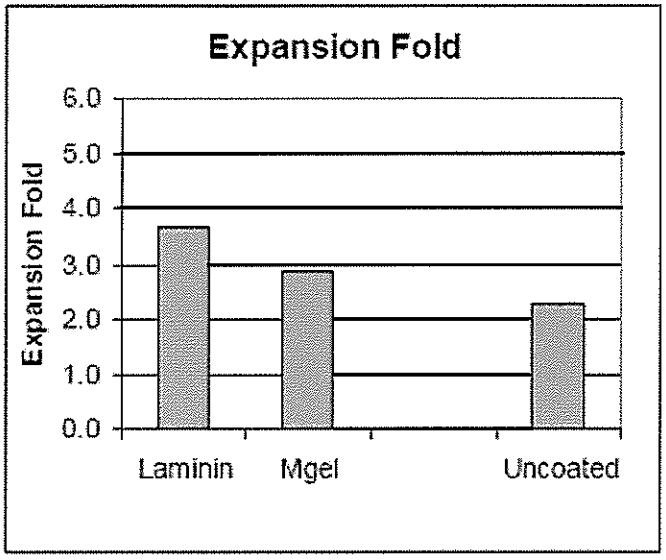


Figure 169

Label	[Xv] Seeding (e6/well)	[DE53] (mg/well)	Medium
1	3	15	bSFS (Control)
2	3	15	bSFS + Hy-Soy (0.1%)
3	3	15	bSFS + Lip Mixture (1x)
4	3	15	bSFS + BSA (1%)
5	3	15	bSFS + Lip Mixture (1x) + Hy-Soy
6	3	15	bSFS + Lip Mixture (1x) + Hy-Soy + BSA

Figure 170

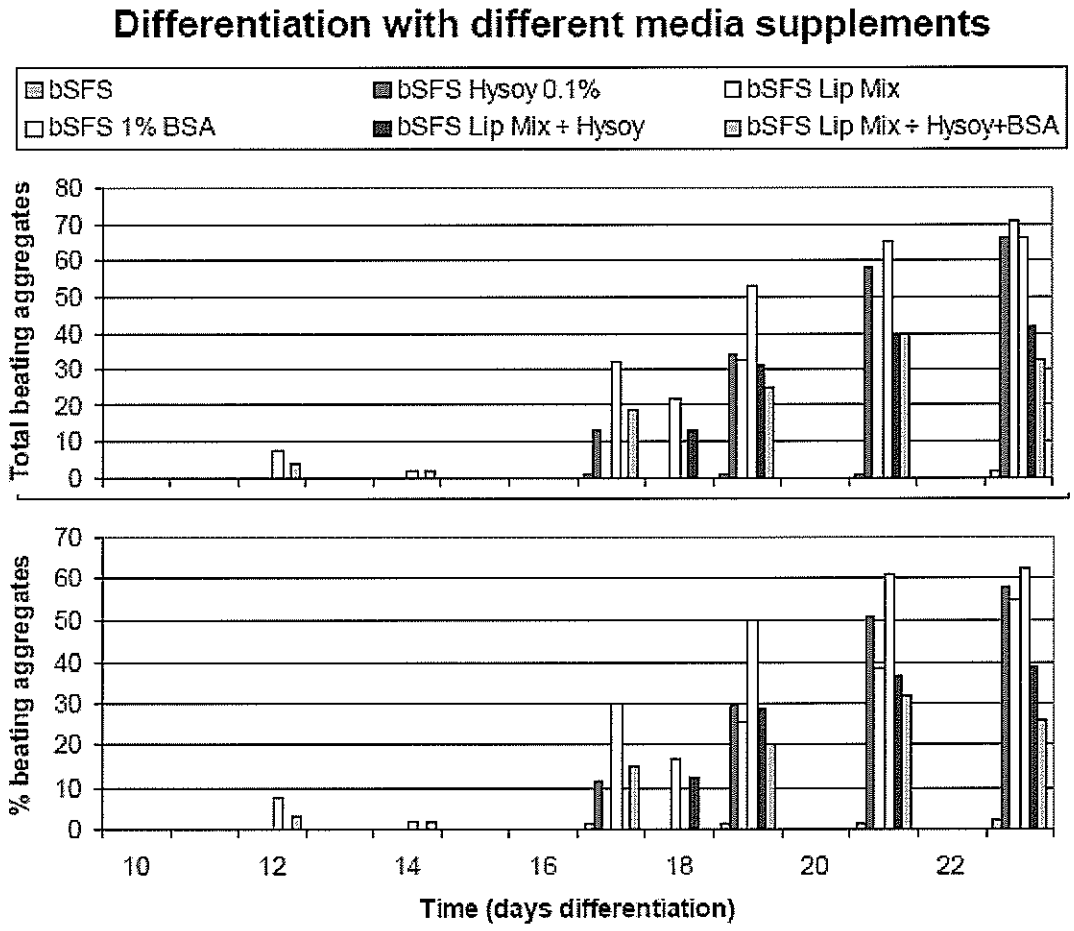

Differentiation with different media supplements
bSFS
bSFS Hysoy 0.1%
bSFS Lip Mix
bSFS 1% BSA
bSFS Lip Mix + Hysoy
bSFS Lip Mix + Hysoy+BSA
Total beating aggregates
% beating aggregates
Time (days differentiation)

Figure 171

Differentiation with different media supplements with hESC seeded from microcarriers to microcarriers

Different additives added to serum free media bSFS or DMEM/F12 + SB203580 (5μM) for differentiation on microcarriers

A) BSFS

B) BSFS + Lipid mixture.

C) DMEM/F12 (with Glutamax, glucose, without insulin, transferrin, selenite).

D) DMEM/F12 (with Glutamax, glucose, without insulin, transferrin, selenite) + Lipid Mixture.

E) BSFS + BSA (0.1%).

F) BSFS + Hy-Soy (0.1%).

Figure 172

Differentiation with different media supplements with hESC seeded from microcarriers to microcarriers

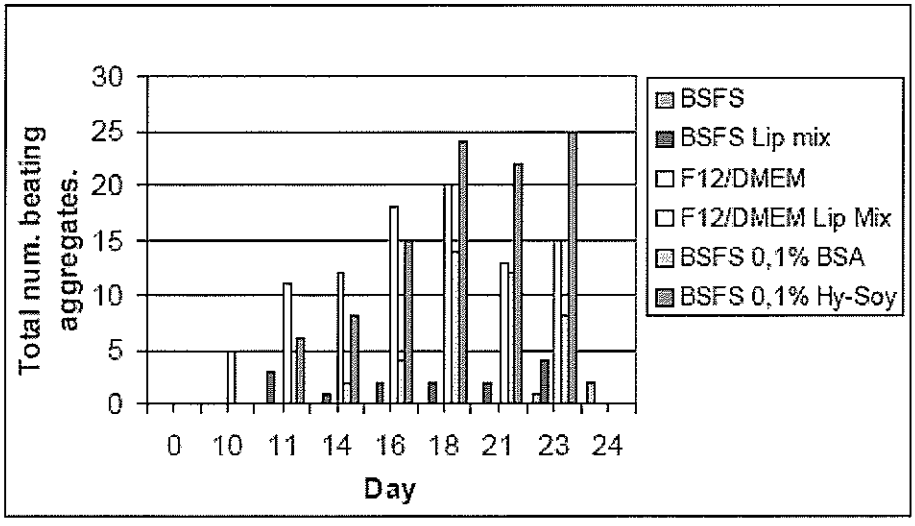


Figure 173

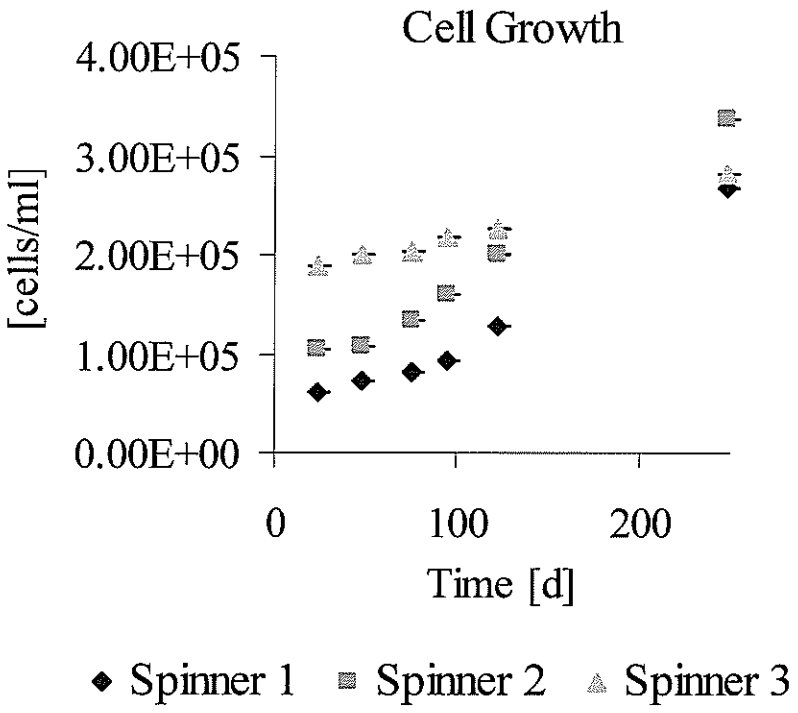

Cell Growth
4.00E+05
3.00E+05
2.00E+05
1.00E+05
0.00E+00
[cells/ml]
0
100
200
Time [d]
Spinner 1
Spinner 2
Spinner 3

Figure 174

	Spinner 1	Spinner 2	Spinner 3
[mg] Carriers/ml	**1.5**	**3**	**5**
no of cells/carrier	12	12	12
Cells/ml	6.0E+04	1.0E+05	1.8E+05
Cells/cm^2	1.3E+04	1.3E+04	1.3E+04
Doubling Time [h]	**103**	**126**	**385**

Figure 175

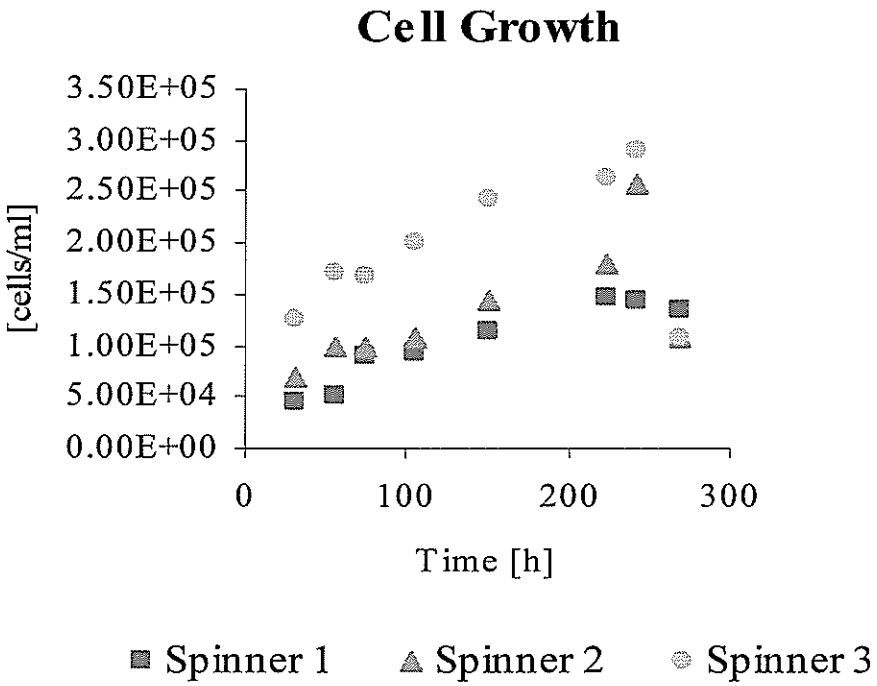


Figure 176

	Spinner 1	Spinner 2	Spinner 3
[mg] Carriers/ml	3.0	3.0	3.0
no of cells/carrier	**5**	**8**	**14**
Cells/ml	4.5E+04	7.2E+04	1.3E+05
Cells/cm^2	5.6E+03	8.9E+03	1.6E+04
Doubling Time [h]	**116**	**133**	**204**

Figure 177

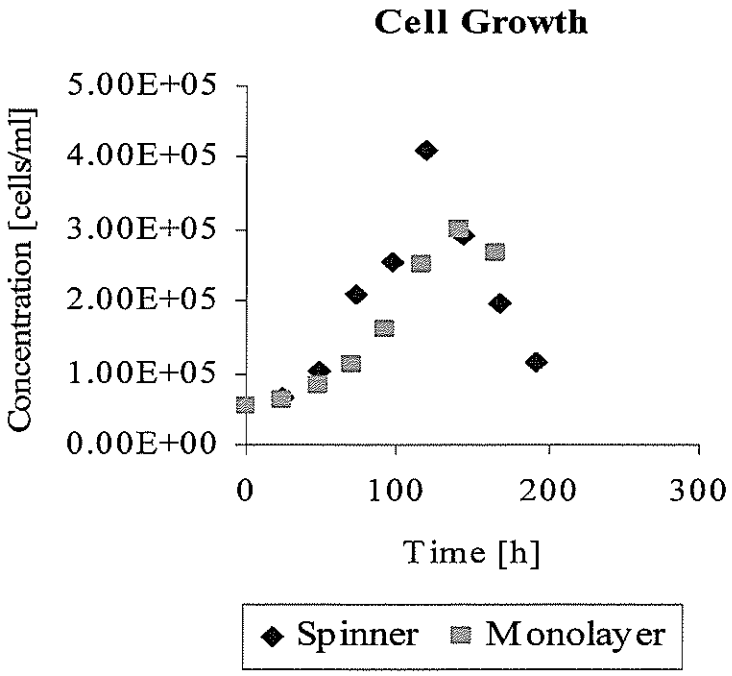


Figure 178

	Monolayer	Spinner
[mg] Microcarriers/ml	NA	3,0
No. Of cells/Carrier	NA	7,5
cells/ml	5.57E4	6.75E4
cells/cm^2	7.00E3	8.30E3
Doubling Time [h]	47	37

Figure 179

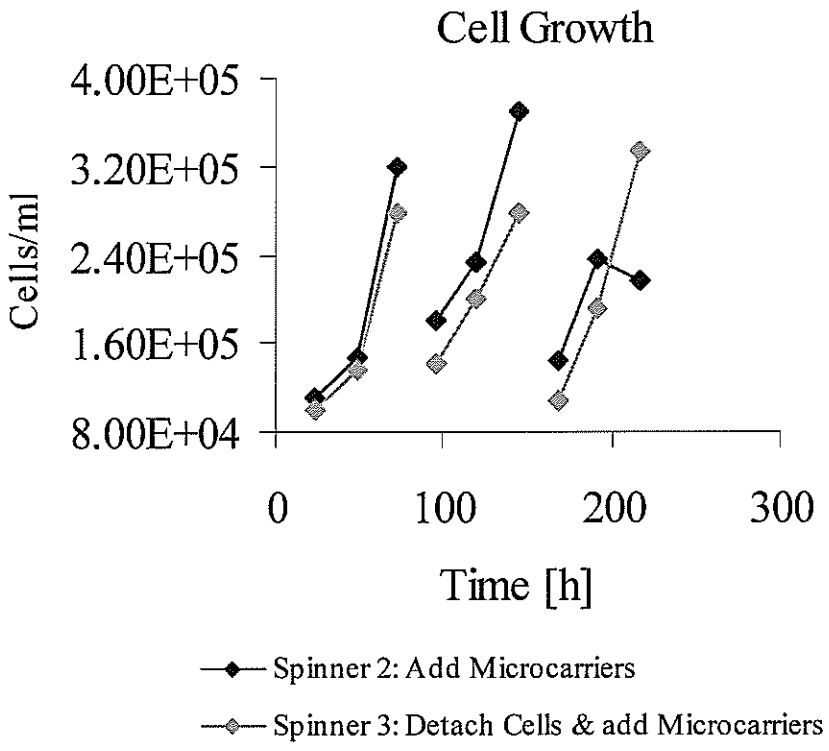


Figure 180

	Doubling Times [h]	
	Spinner 2	**Spinner 3**
Passage 1	31.08	32.54
Passage 2	45.90	48.81
Passage 3	81.55	29.37

Figure 181

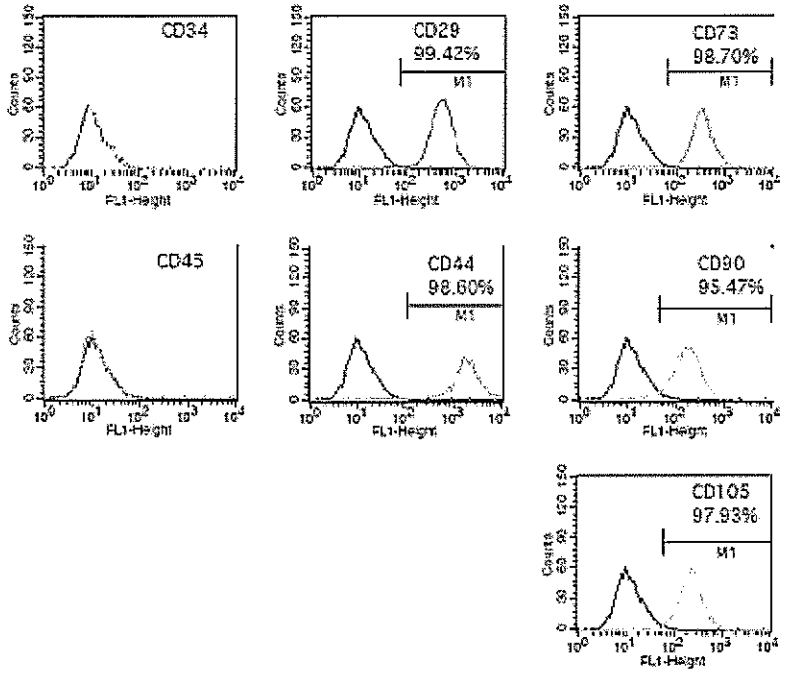


Figure 182

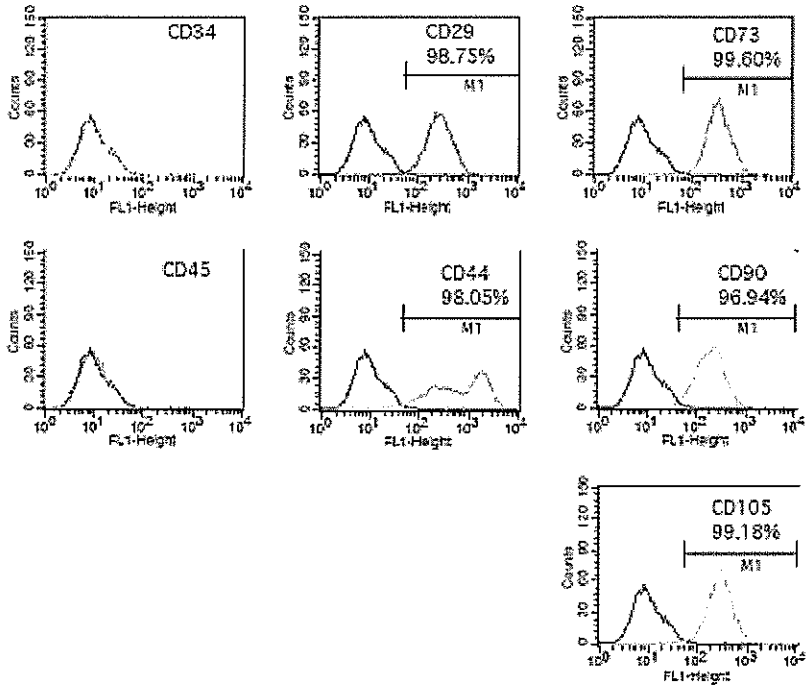


Figure 183

ERSATZBLATT (REGEL 26)

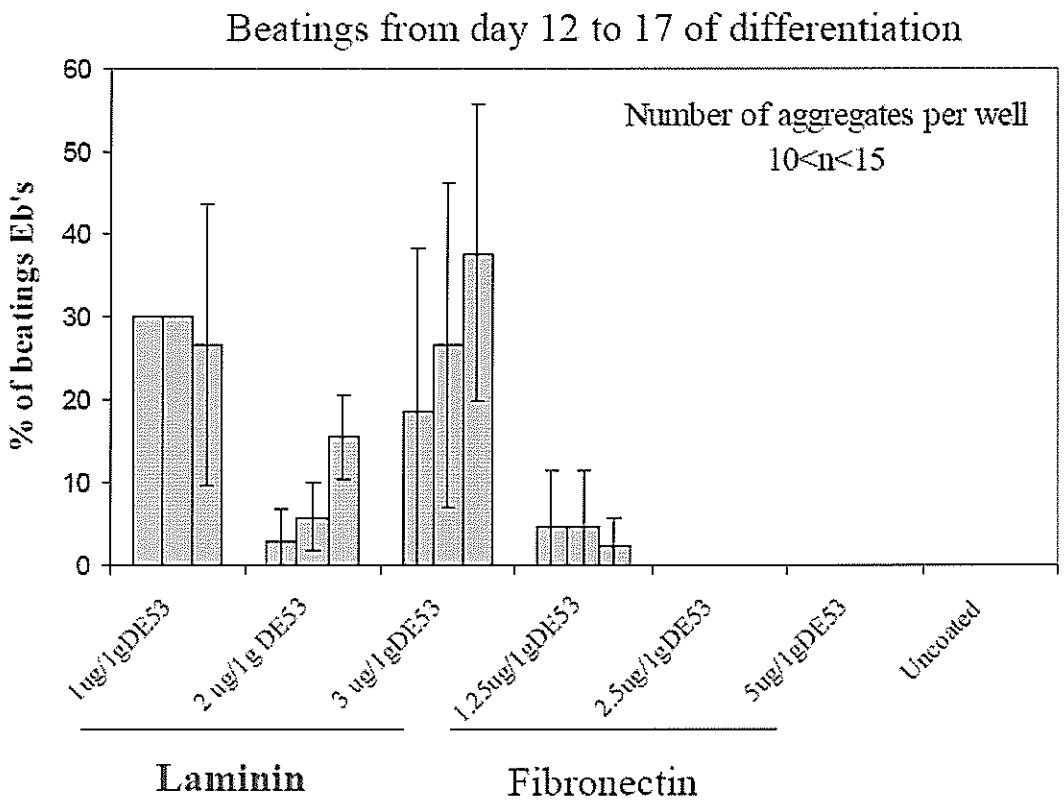

Beatings from day 12 to 17 of differentiation
Number of aggregates per well
10<n<15
% of beatings Eb's
60
50
40
30
20
10
0
1ug/1gDE53
2 ug/1g DE53
3 ug/1gDE53
1.25ug/1gDE53
2.5ug/1gDE53
5ug/1gDE53
Uncoated
Laminin
Fibronectin

Figure 184

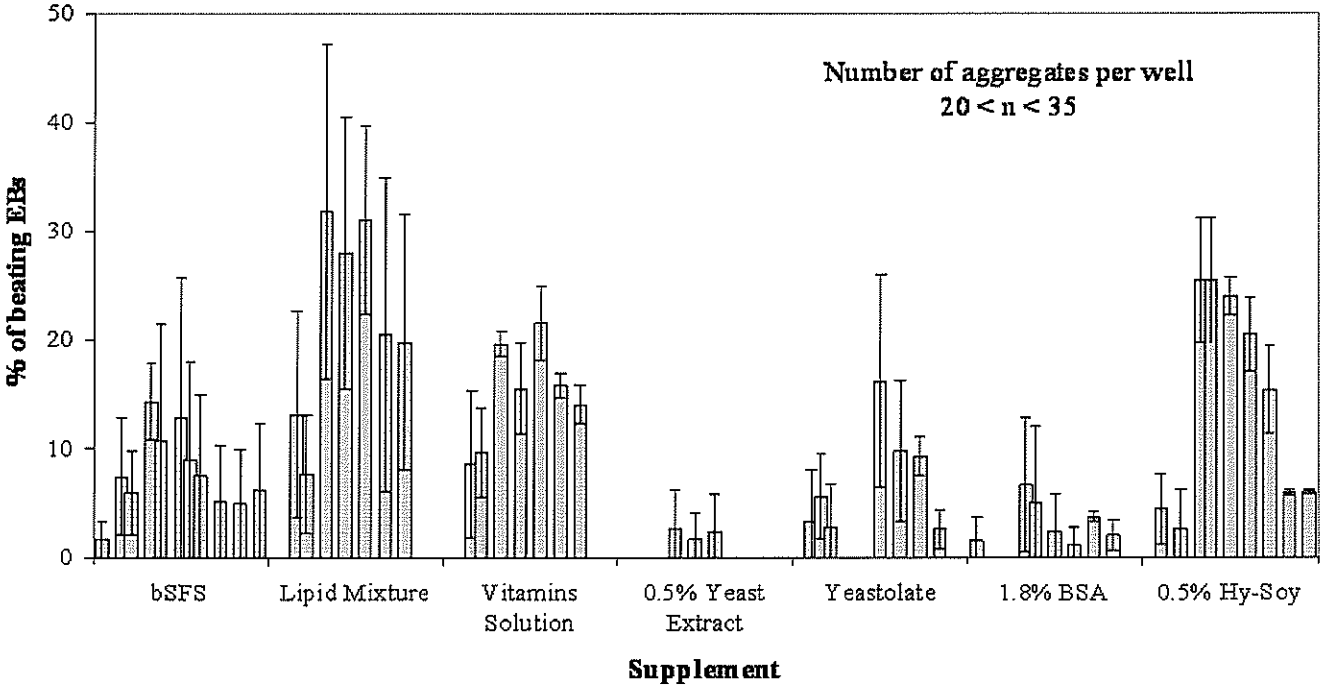

Number of aggregates per well
20 < n < 35
% of beating EBs
50
40
30
20
10
0
bSFS
Lipid Mixture
Vitamins Solution
0.5% Yeast Extract
Yeastolate
1.8% BSA
0.5% Hy-Soy
Supplement

Figure 185

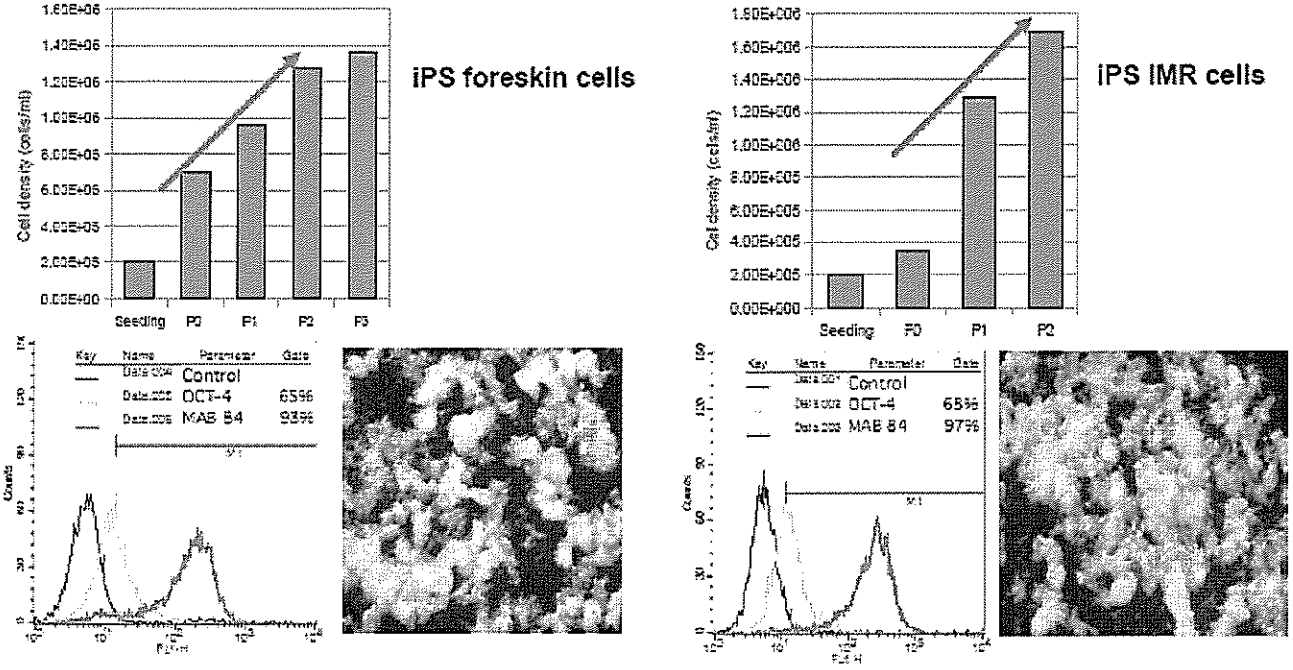

iPS foreskin cells
Cell density (cells/ml)
Seeding
P0
P1
P2
P3
Key
Name
Parameter
Gate
Control
OCT-4
65%
MAB 84
93%
iPS IMR cells
Cell density (cells/ml)
Seeding
P0
P1
P2
Key
Name
Parameter
Gate
Control
OCT-4
65%
MAB 84
97%

Figure 186

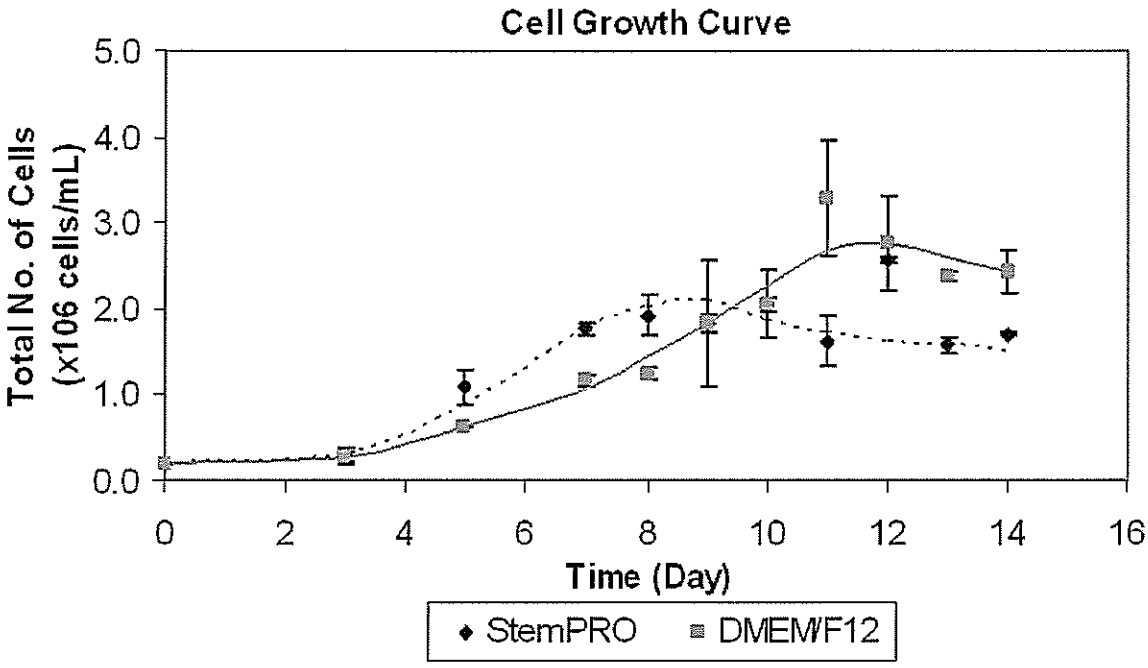

Cell Growth Curve
Total No. of Cells (x106 cells/mL)
5.0
4.0
3.0
2.0
1.0
0.0
0
2
4
6
8
10
12
14
16
Time (Day)
StemPRO
DMEM/F12

Figure 187