FIG. 1

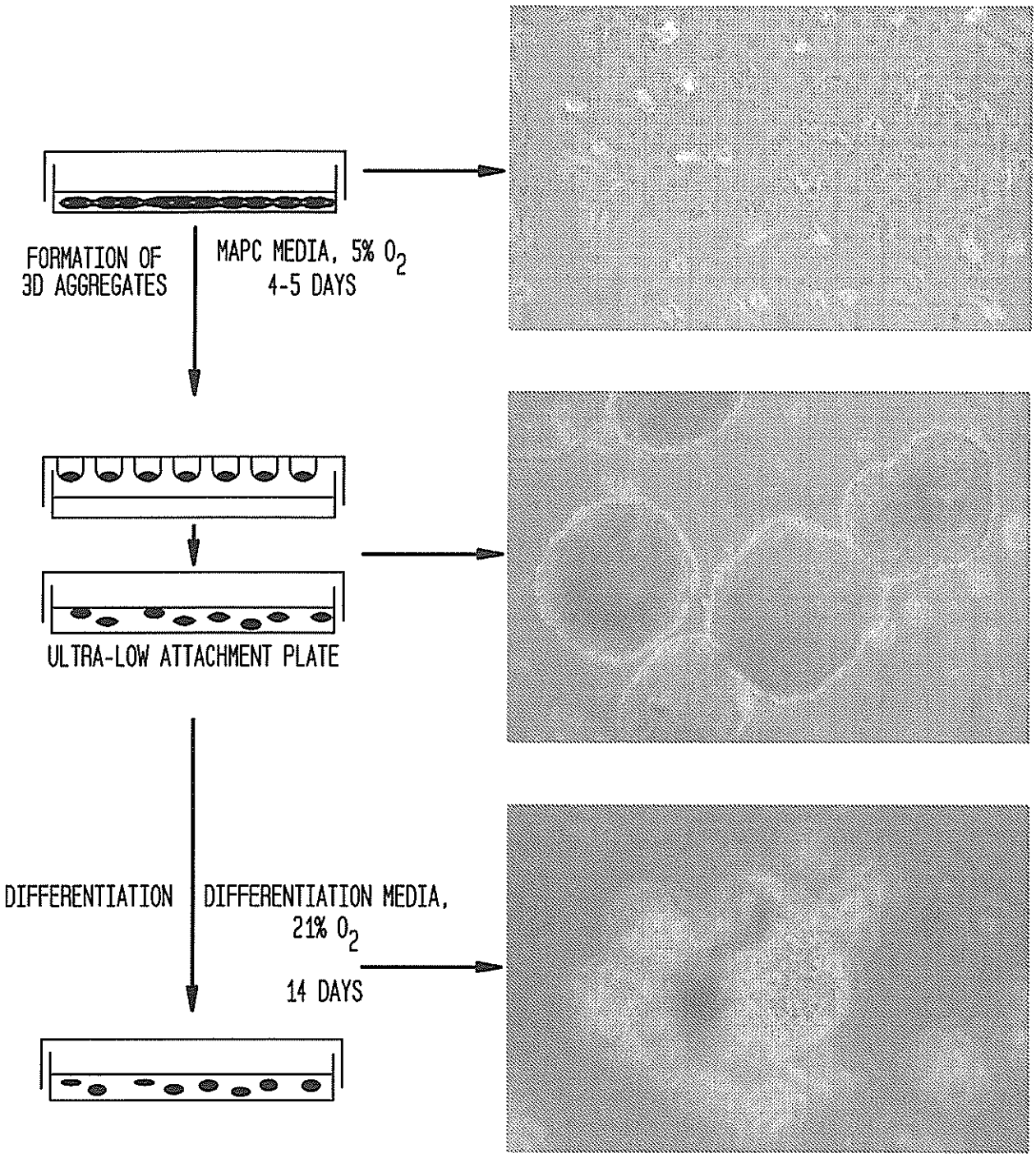

FORMATION OF
3D AGGREGATES
MAPC MEDIA, 5% O_2
4-5 DAYS
ULTRA-LOW ATTACHMENT PLATE
DIFFERENTIATION
DIFFERENTIATION MEDIA,
21% O_2
14 DAYS

FIG. 2

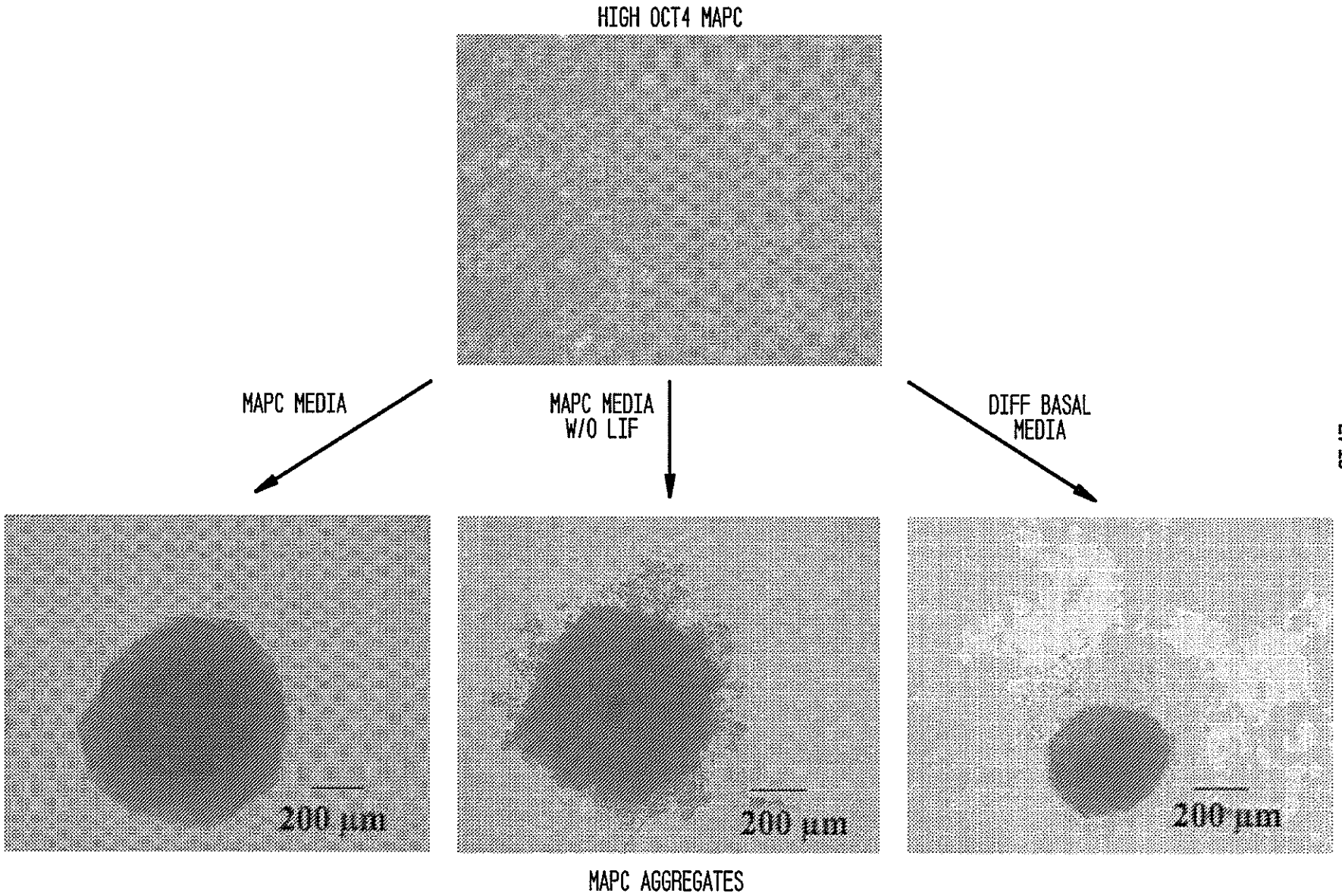

HIGH OCT4 MAPC
MAPC MEDIA
MAPC MEDIA W/O LIF
DIFF BASAL MEDIA
200 µm
200 µm
200 µm
MAPC AGGREGATES

FIG. 3

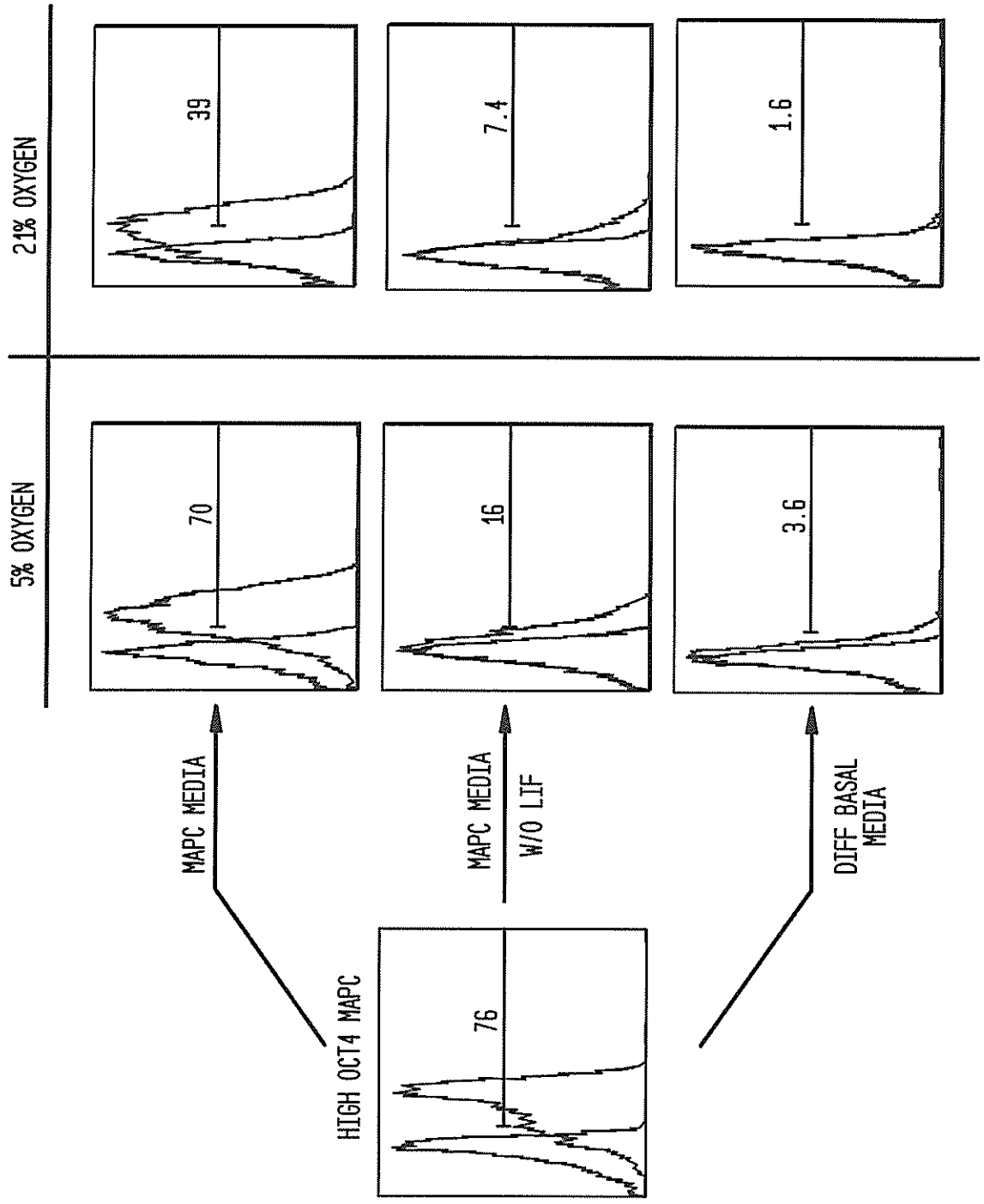

21% OXYGEN
5% OXYGEN
39
7.4
1.6
70
16
3.6
76
MAPC MEDIA
MAPC MEDIA
W/O LIF
DIFF BASAL
MEDIA
HIGH OCT4 MAPC

FIG. 4

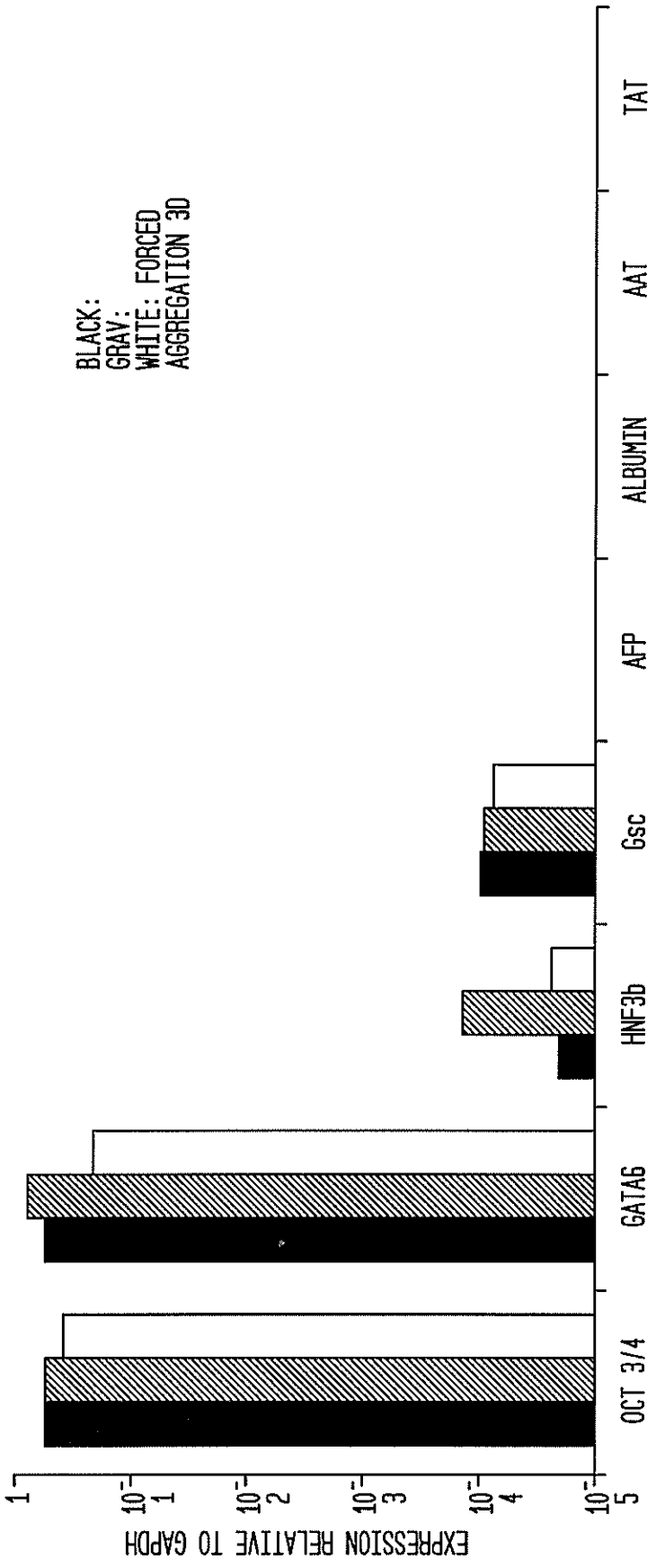
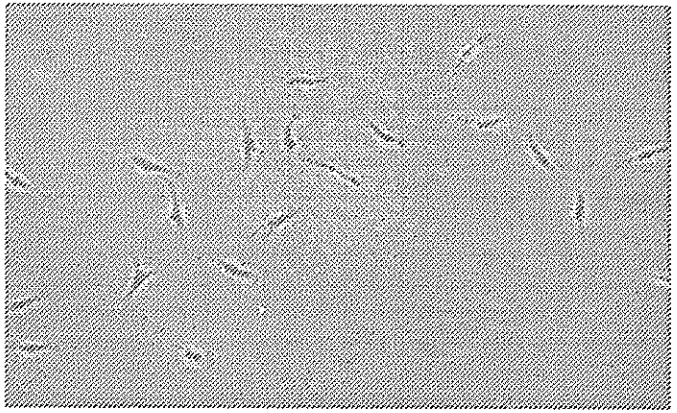

BLACK:
GRAY:
WHITE: FORCED AGGREGATION 3D
EXPRESSION RELATIVE TO GAPDH
1
10-1
10-2
10-3
10-4
10-5
OCT 3/4
GATA6
HNF3b
Gsc
AFP
ALBUMIN
AAT
TAT

FIG. 5

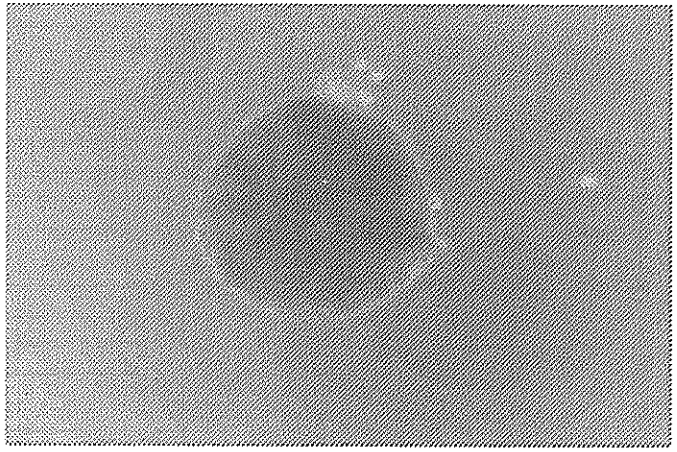
LOW OCT4 MAPC



MAPC MEDIA, 5% O_2

- LOW OCT4 MAPC AGGREGATES WERE FORMED FROM 8000 CELLS AND TOOK 7 DAYS TO FORM.
- SIZE: ~150-200 μm
- DIFFERENTIATED SPONTANEOUSLY TO DIFFERENT CELL TYPES

LOW OCT4 MAPC AGGREGATE



DIFF BASAL MEDIA, 21% O_2

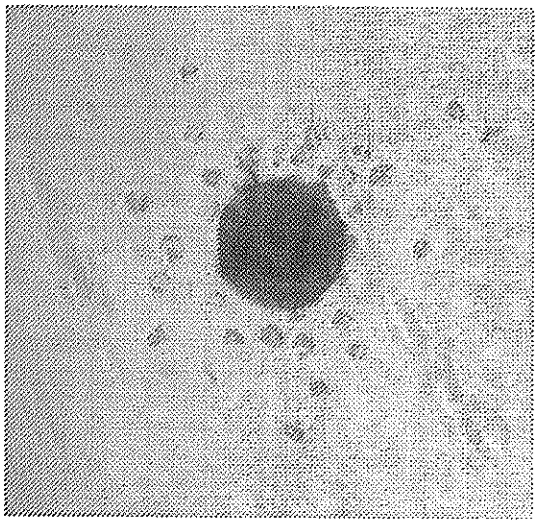


FIG. 6

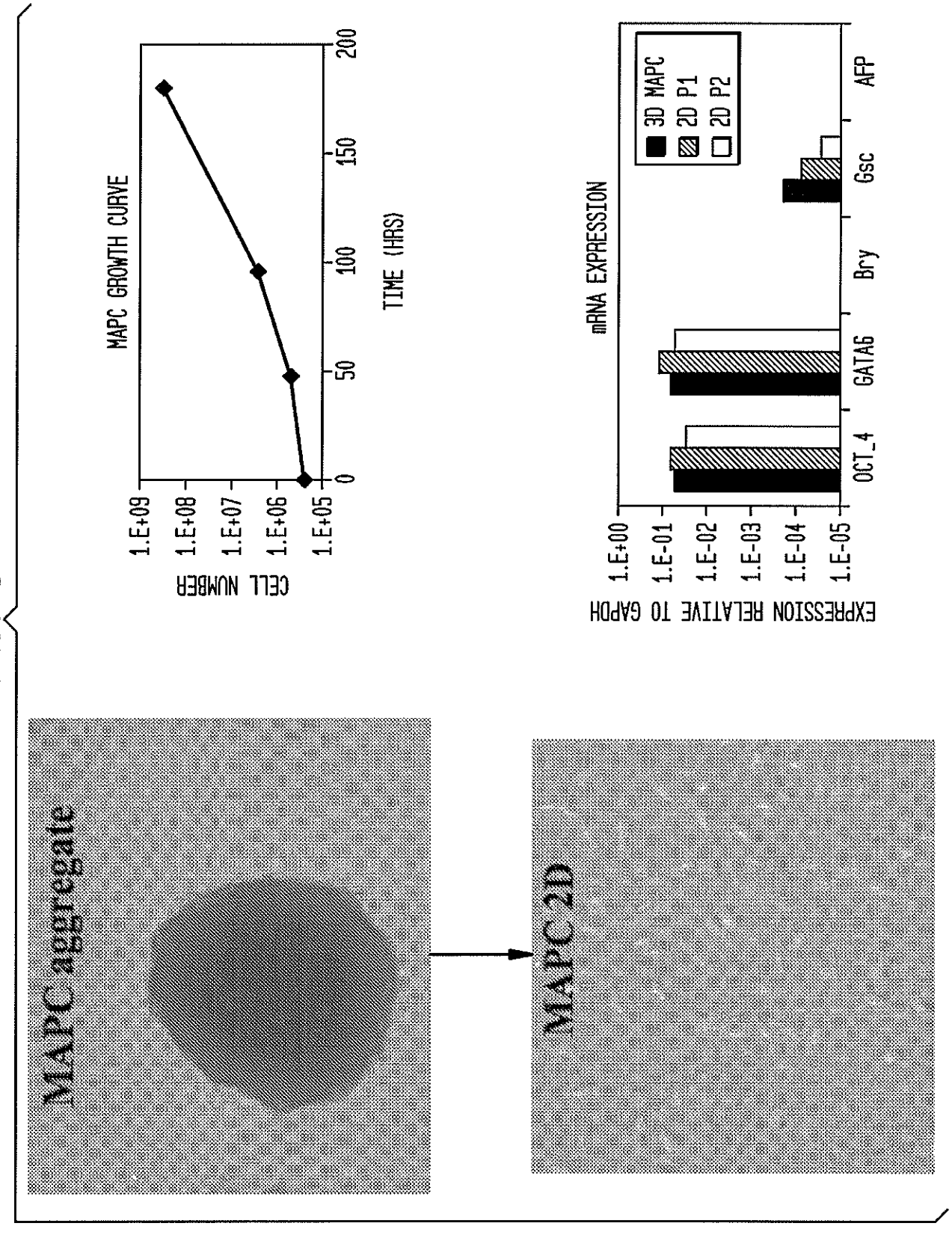

MAPC aggregate
MAPC 2D
MAPC GROWTH CURVE
CELL NUMBER
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
0
50
100
150
200
TIME (HRS)
mRNA EXPRESSION
3D MAPC
2D P1
2D P2
EXPRESSION RELATIVE TO GAPDH
1.E+00
1.E-01
1.E-02
1.E-03
1.E-04
1.E-05
OCT_4
GATA6
Bry
Gsc
AFP

FIG. 7

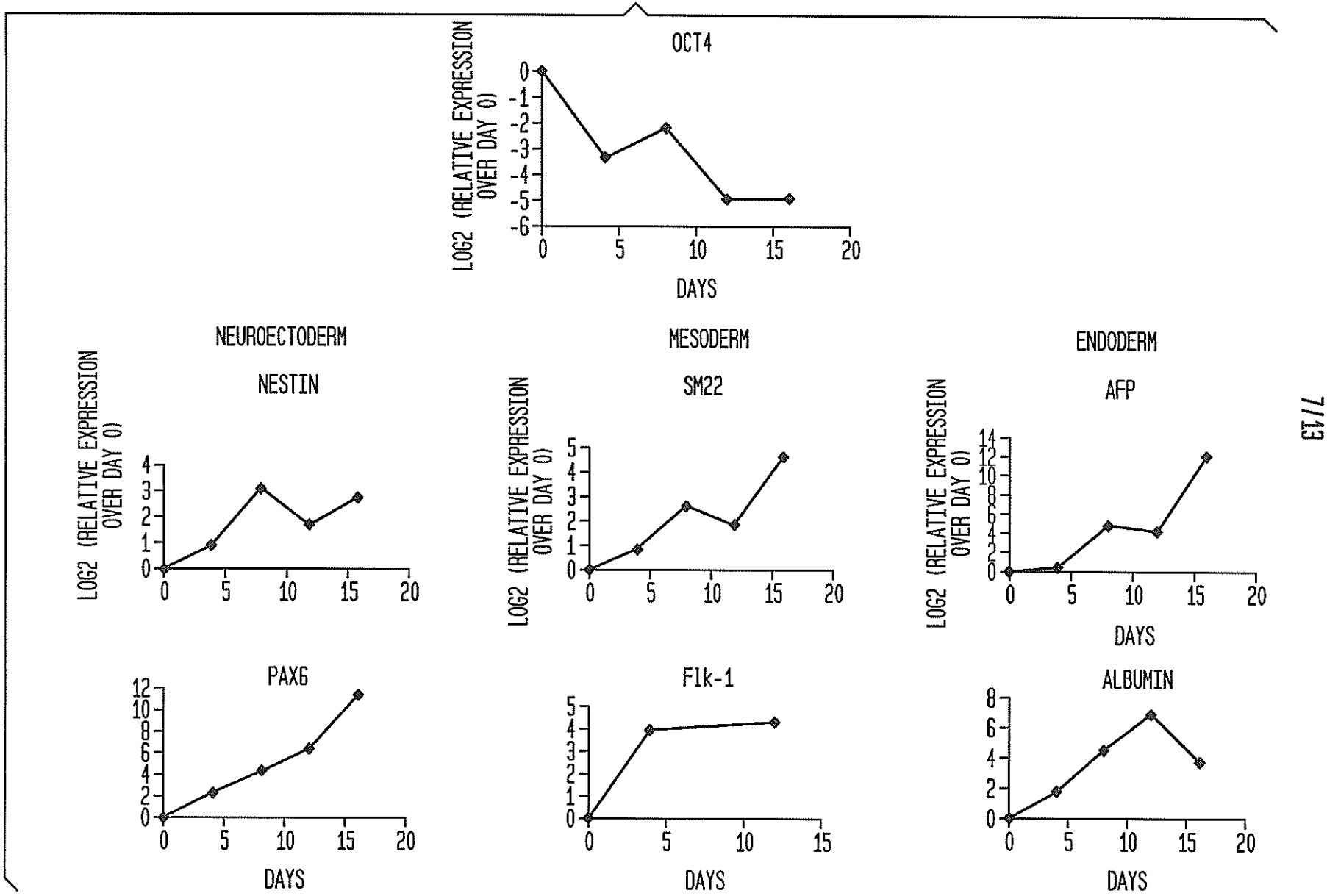

OCT4
LOG2 (RELATIVE EXPRESSION OVER DAY 0)
0
-1
-2
-3
-4
-5
-6
0
5
10
15
20
DAYS
NEUROECTODERM
NESTIN
LOG2 (RELATIVE EXPRESSION OVER DAY 0)
4
3
2
1
0
0
5
10
15
20
PAX6
12
10
8
6
4
2
0
0
5
10
15
20
DAYS
MESODERM
SM22
LOG2 (RELATIVE EXPRESSION OVER DAY 0)
5
4
3
2
1
0
0
5
10
15
20
Flk-1
5
4
3
2
1
0
0
5
10
15
DAYS
ENDODERM
AFP
LOG2 (RELATIVE EXPRESSION OVER DAY 0)
14
12
10
8
6
4
2
0
0
5
10
15
20
DAYS
ALBUMIN
8
6
4
2
0
0
5
10
15
20
DAYS

FIG. 8

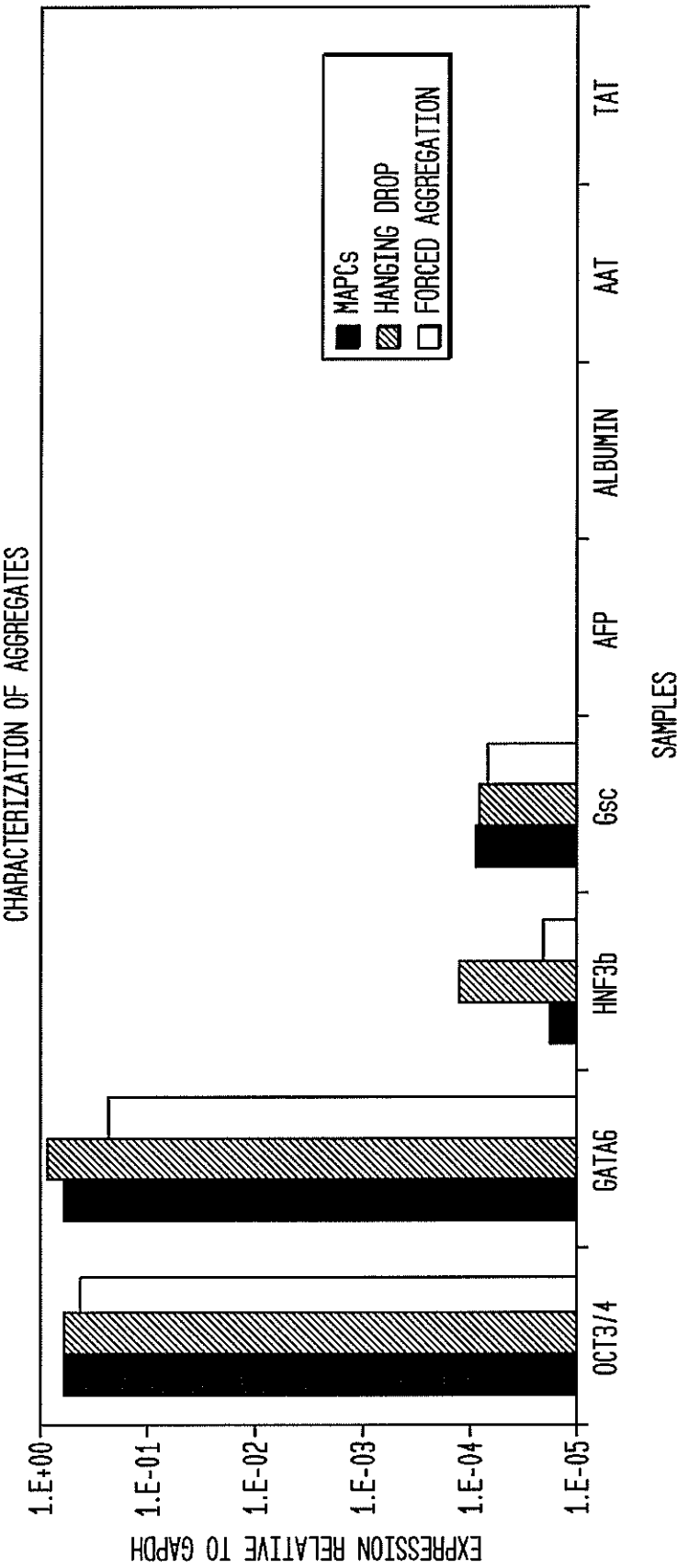

CHARACTERIZATION OF AGGREGATES
MAPCs
HANGING DROP
FORCED AGGREGATION
EXPRESSION RELATIVE TO GAPDH
1.E+00
1.E-01
1.E-02
1.E-03
1.E-04
1.E-05
OCT3/4
GATA6
HNF3b
Gsc
AFP
ALBUMIN
AAT
TAT
SAMPLES

FIG. 9

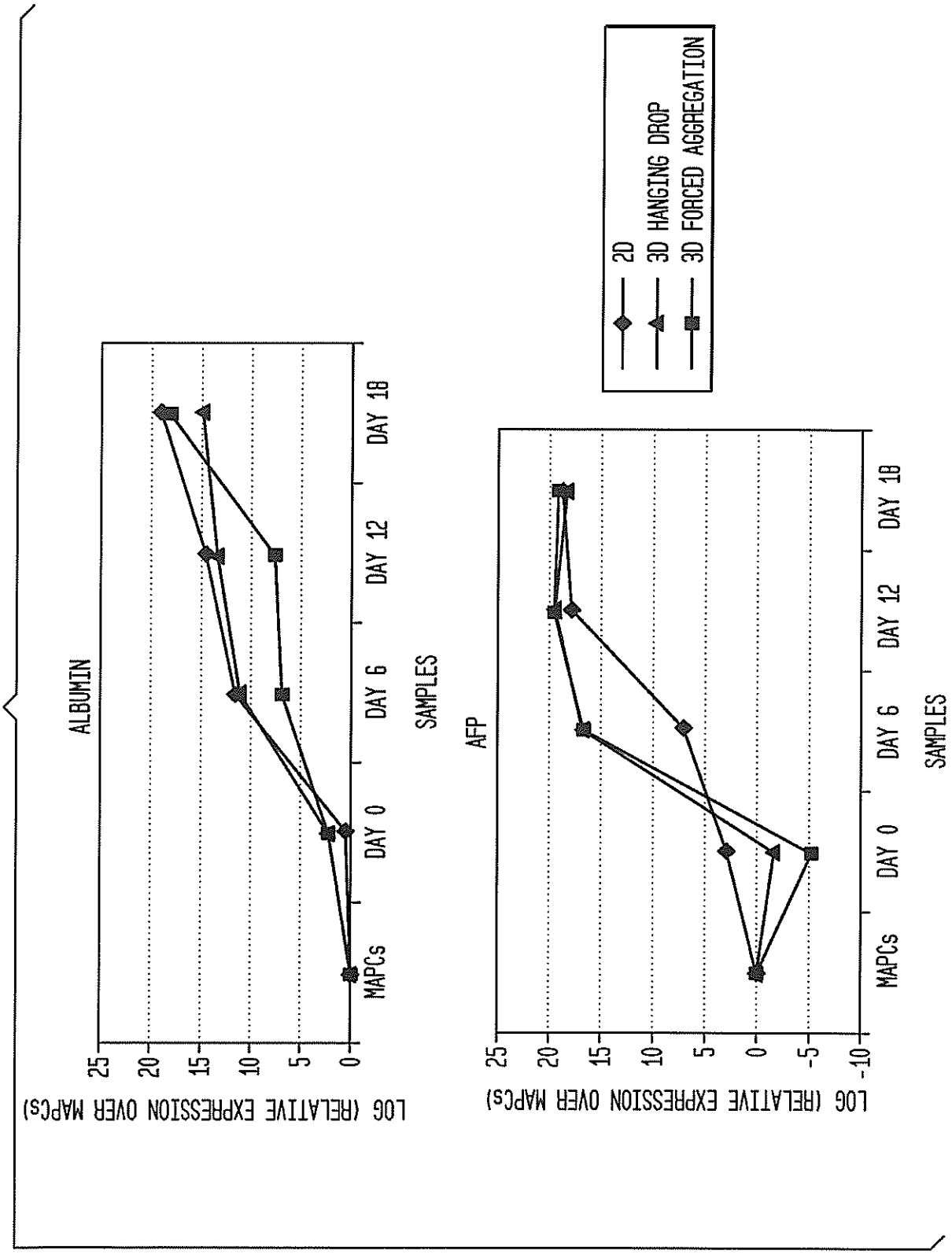

ALBUMIN
LOG (RELATIVE EXPRESSION OVER MAPCs)
25
20
15
10
5
0
MAPCs
DAY 0
DAY 6
DAY 12
DAY 18
SAMPLES
AFP
LOG (RELATIVE EXPRESSION OVER MAPCs)
25
20
15
10
5
0
-5
-10
MAPCs
DAY 0
DAY 6
DAY 12
DAY 18
SAMPLES
2D
3D HANGING DROP
3D FORCED AGGREGATION

FIG. 10

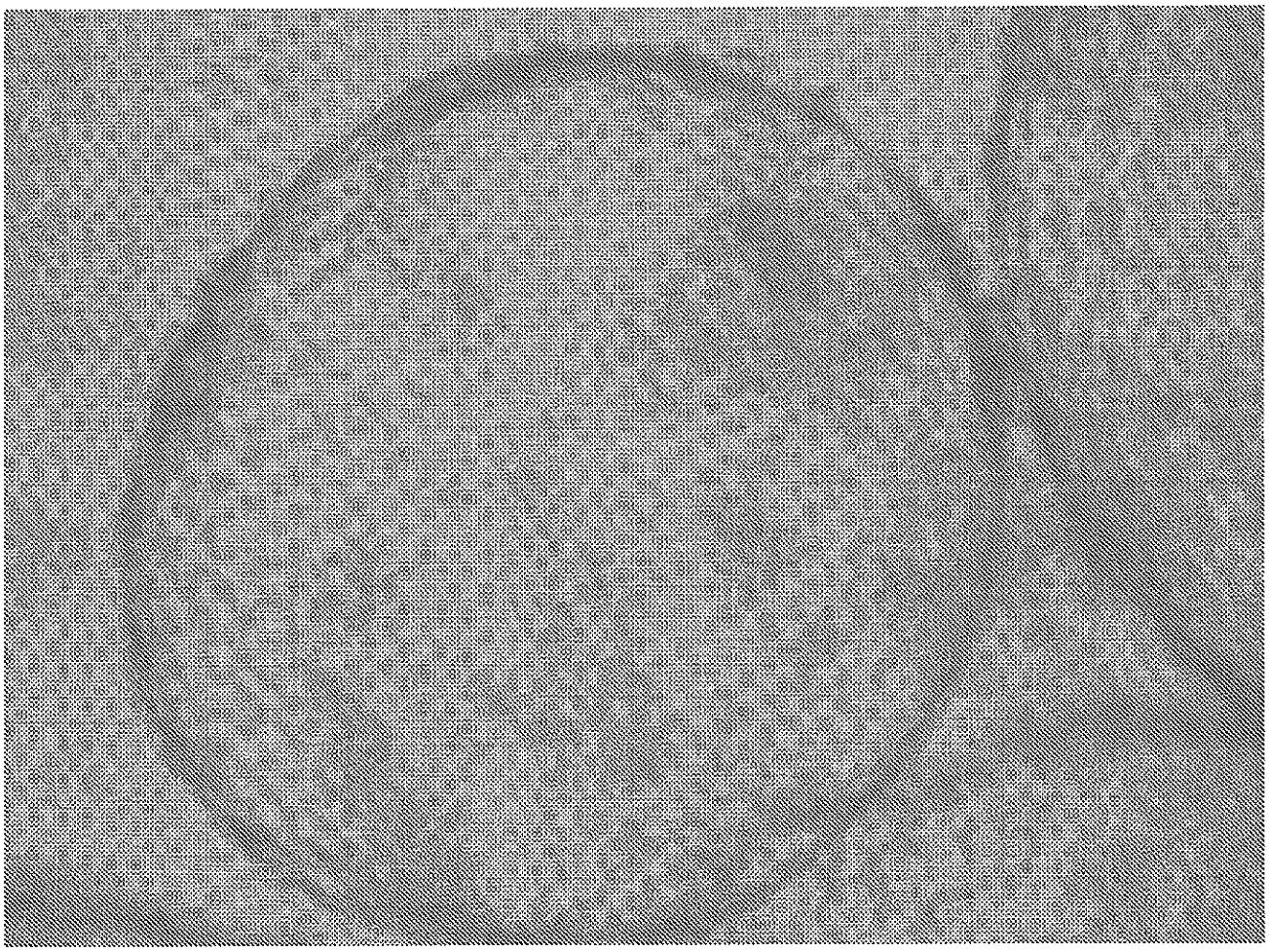


FIG. 11A

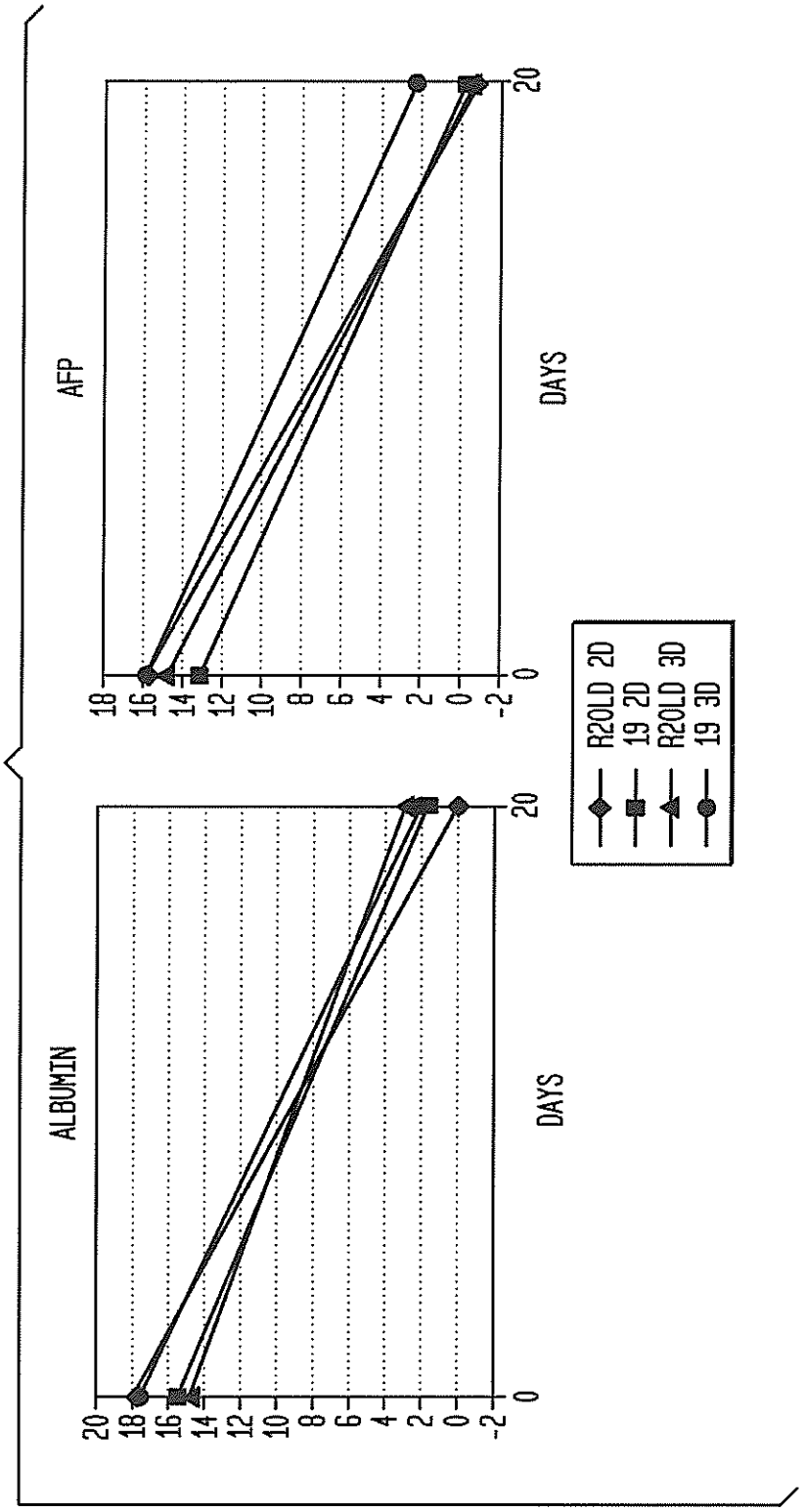


FIG. 11B

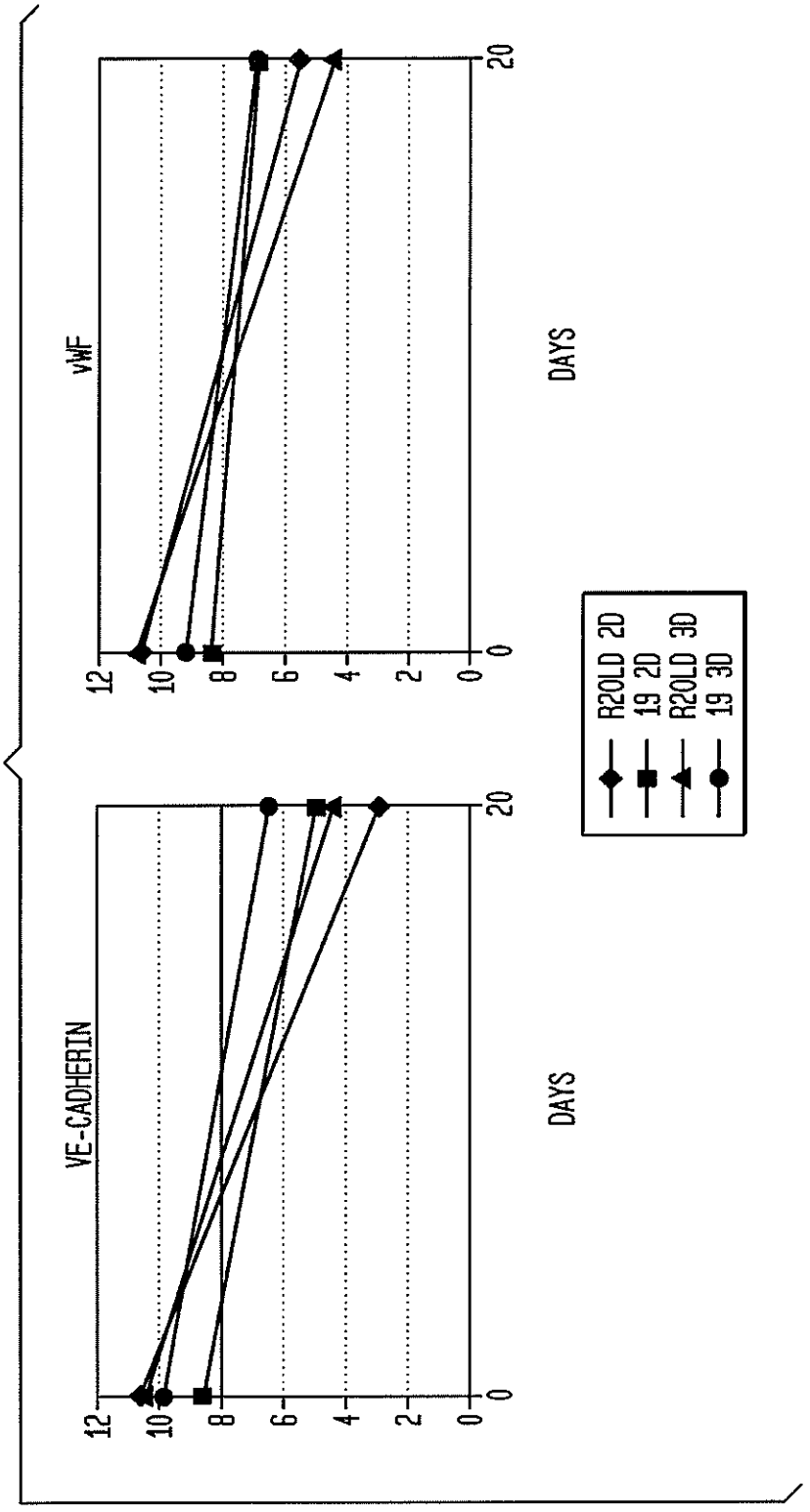
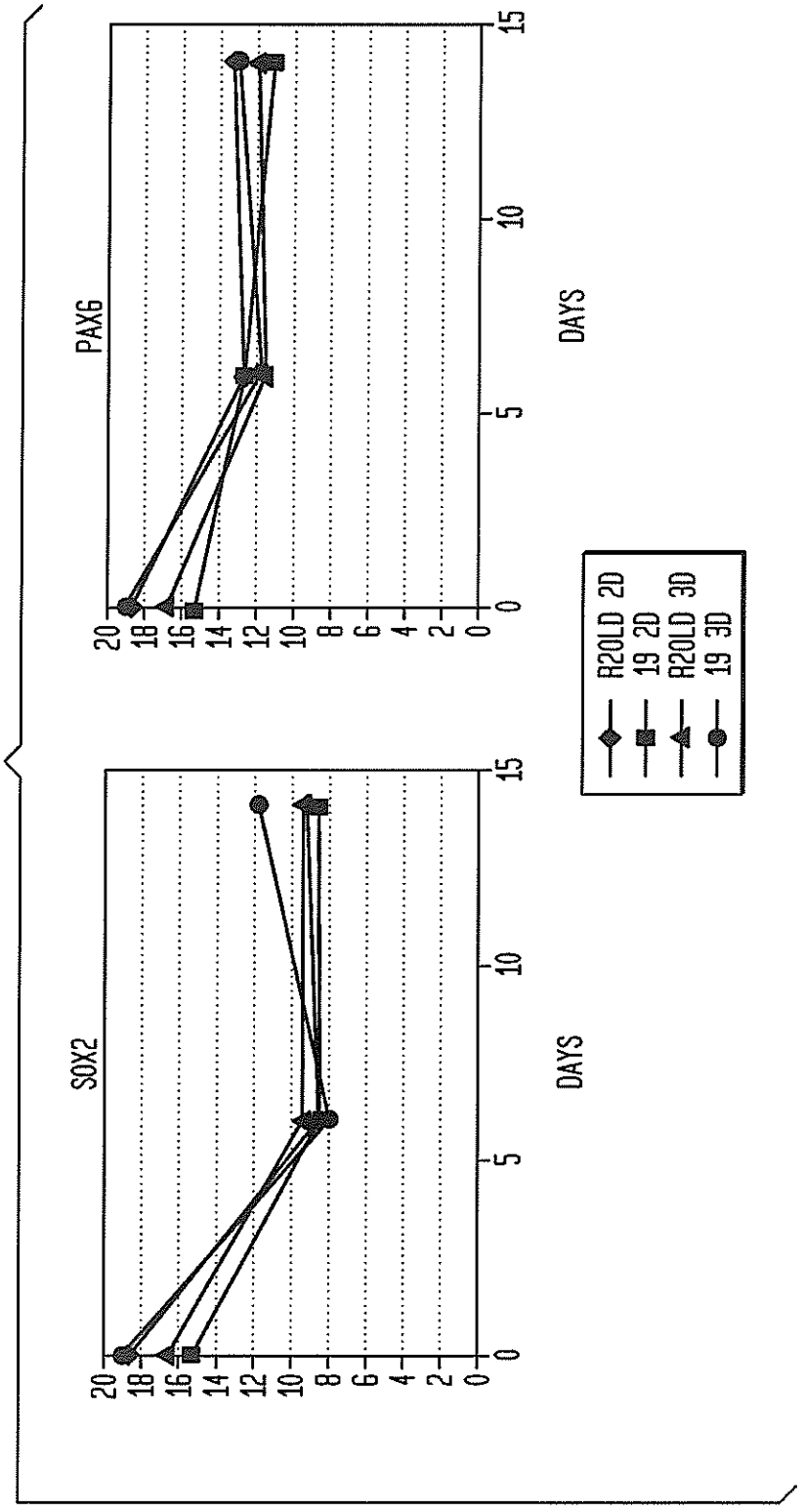

VE-CADHERIN
VWF
DAYS
DAYS
0
2
4
6
8
10
12
0
20
R20LD 2D
19 2D
R20LD 3D
19 3D

FIG. 11C


SOX2
PAX6
DAYS
DAYS
0
2
4
6
8
10
12
14
16
18
20
0
5
10
15
R20LD 2D
19 2D
R20LD 3D
19 3D