passages had no significant differences. Data presented as mean ± SD from three independent experiments. (B) Fluorescence micrographs of colonies of hESCs cultured on grafted PMEDSAH and ATRP PMEDSAH showing expression of pluripotent markers after 5 passages. Primary antibodies OCT4 and SSEA-4 were used to detect the expression of these markers from hESCs cultured on grafted PMEDSAH and ATRP PMEDSAH (25nm, 105nm and 176nm). hESC cultured on 105nm ATRP PMEDSAH after 5 passages (C) showed expression of pluripotent markers in fluorescence micrographs and (D) kept a normal karyotype.

Fig. 4. Modified PMEDSAH supports hESC pluripotency. hESCs cultured on 105nm ATRP PMEDSAH after 5 passages retained pluripotency as demonstrated by induced specific lineage differentiation with expression of genes representing different germ layers. Data presented in as mean ± SD from three independent experiments .

Table 1 Calculated total cell number of hESCs cultured on different substrates

Table 2 EB formation with expression of genes representing different germ layers. EBs were formed from hESCs cultured on 105nm ATRP PMEDSAH after 5 passages. Quantitative RT-PCR showed increasing transcript levels of genes representing different germ layers from EBs compared to the undifferentiated hESCs grown on 105 nm ATRP PMEDSAH after 5 passages.

Supplementary Fig. 1. Gel architecture influences the undifferentiated colony formation of hESCs (H9 cells). Plot of undifferentiated colony number ratio compared to Matrigel indicated ATRP PMEDSAH with a 105nm hydrogel thickness led to a higher number of undifferentiated colonies compared to other experimental groups. Plot of undifferentiated colony area indicated no significant differences among cells cultured on different polymer

substrates. Data presented as mean ± SD from three independent experiments (* $p<0.05$).

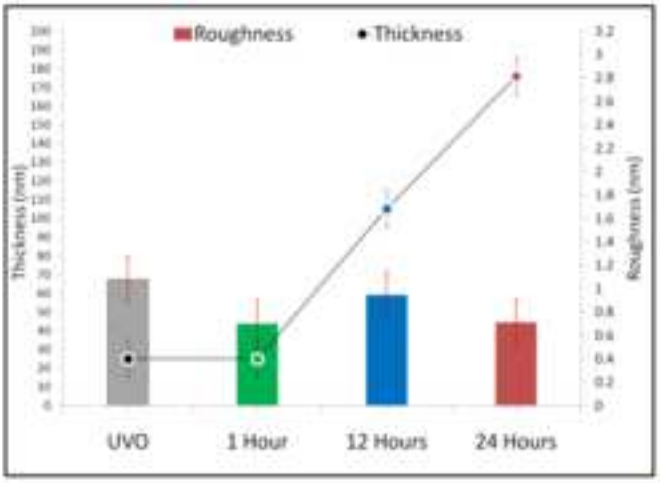
Supplementary Fig. 2. Expression of SSEA-4 in hESCs cultured on different substrates after multiple passages. hESCs cultured on different substrates from week 1 to week 5 were analyzed by flow cytometry to determine the percentage of SSEA-4 expressing cells. Background fluorescence and autofluorescence were determined using cells without treatment (- Control) and treated with Mouse IgG1 Phycoerythrin Isotype Control (PE). Because cells grown on Matrigel decreased after each passage, the amount of cells was not enough for flow cytometry from week 3.

Substrates	Calculated total cell numbers from Week 0 to Week 5					
	W0	W1	W2	W3	W4	W5
Matrigel	$2x10^4$	$3.7x10^5$	$2.4x10^6$	$3.5x10^6$	$1.2x10^8$	/
Grafted PMEDSAH	$2x10^4$	$1.4x10^5$	$8.2x10^5$	$5.0x10^6$	$2.7x10^7$	$1.4x10^8$
ATRP PMEDSAH (25nm)	$2x10^4$	$2.5x10^5$	$2.4x10^6$	$3.4x10^7$	$2.9x10^8$	$2.9x10^9$
ATRP PMEDSAH (105nm)	$2x10^4$	$3.2x10^5$	$4.0x10^6$	$4.9x10^7$	$4.0x10^8$	$4.7x10^9$
ATRP PMEDSAH (176nm)	$2x10^4$	$1.7x10^5$	$1.4x10^6$	$1.0x10^7$	$6.2x10^7$	$3.7x10^8$

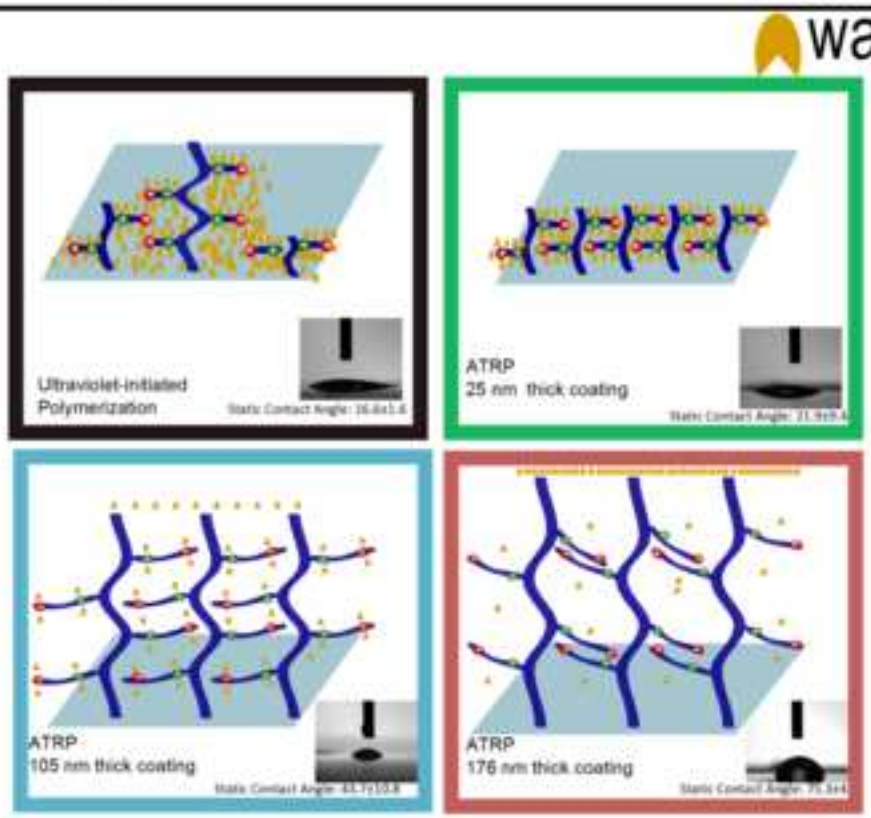
Formula: $CN_{(n+1)} = CN_n \times TN_{(n+1)}/20000$
CN: Calculated total cell number, *TN*: total cell number, *n*: culture week number

	Gene	Relative Transcript Levels ± SD
Endoderm	*PDX1*	35.58 ± 2.58
	AFP	53.14 ± 4.76
	SOX17	159.47 ± 12.51
	FOXA2	249.12 ± 25.67
Mesoderm	*HESX1*	10.21 ± 0.84
	TNN13	94.32 ± 6.99
	NKX2-5	117.31 ± 4.04
Ectoderm	*NES*	3.58 ± 0.15
	SOX1	37.26 ± 1.35
	PAX6	107.36 ± 7.27
	NEUROD1	860.77 ± 97.08

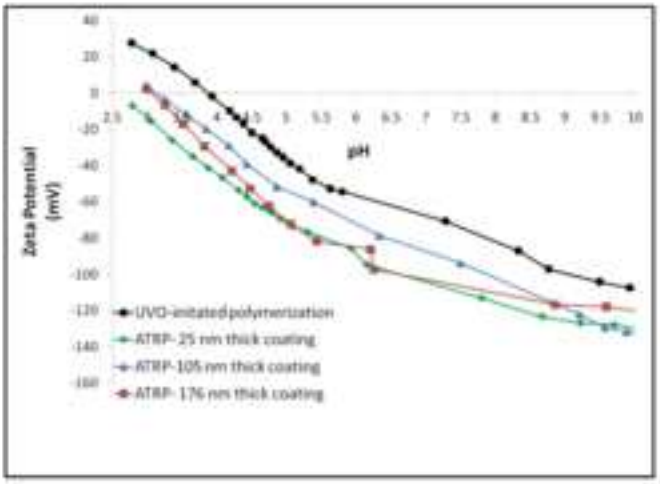
A

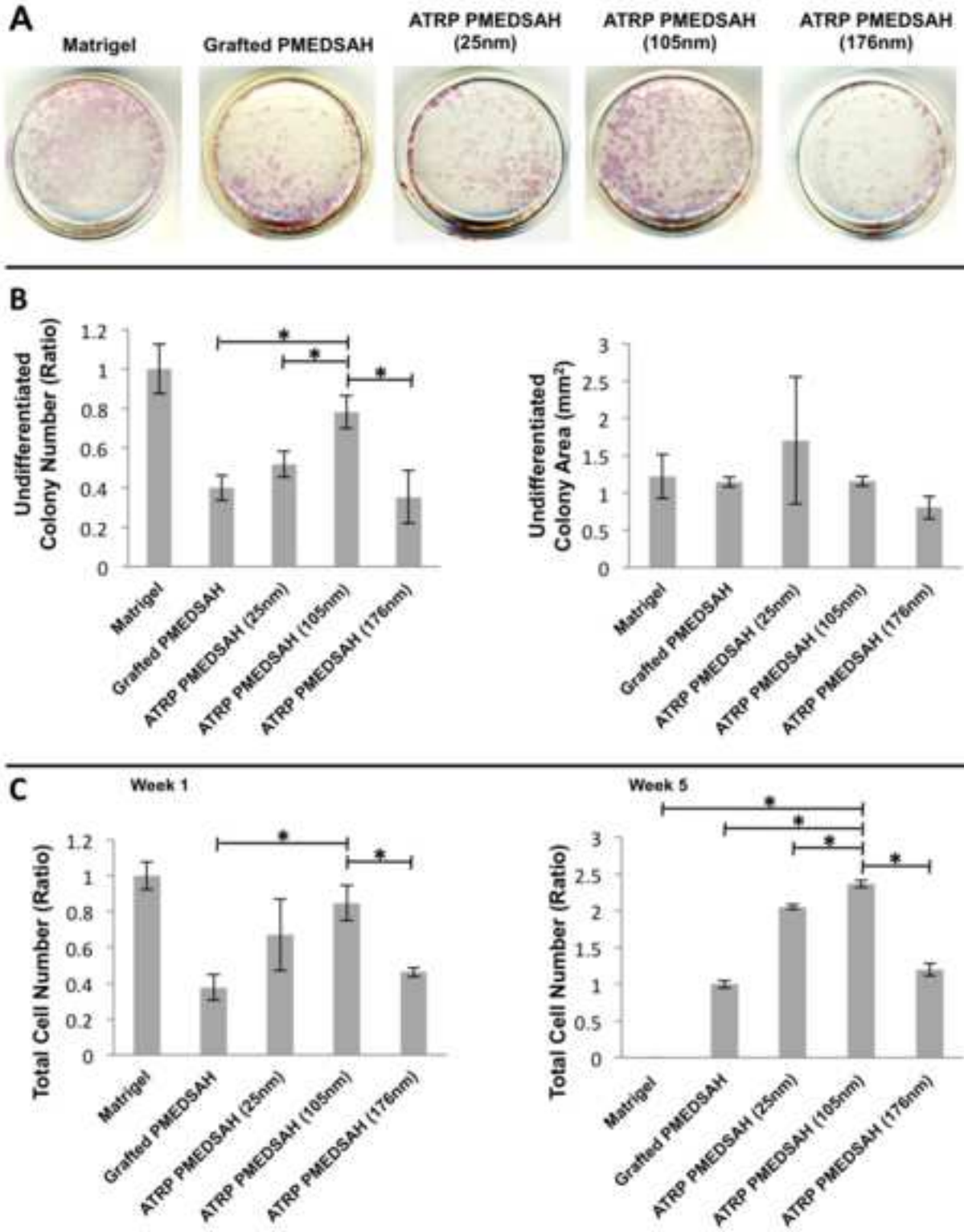

Roughness
Thickness
Thickness (nm)
Roughness (nm)
UVO
1 Hour
12 Hours
24 Hours

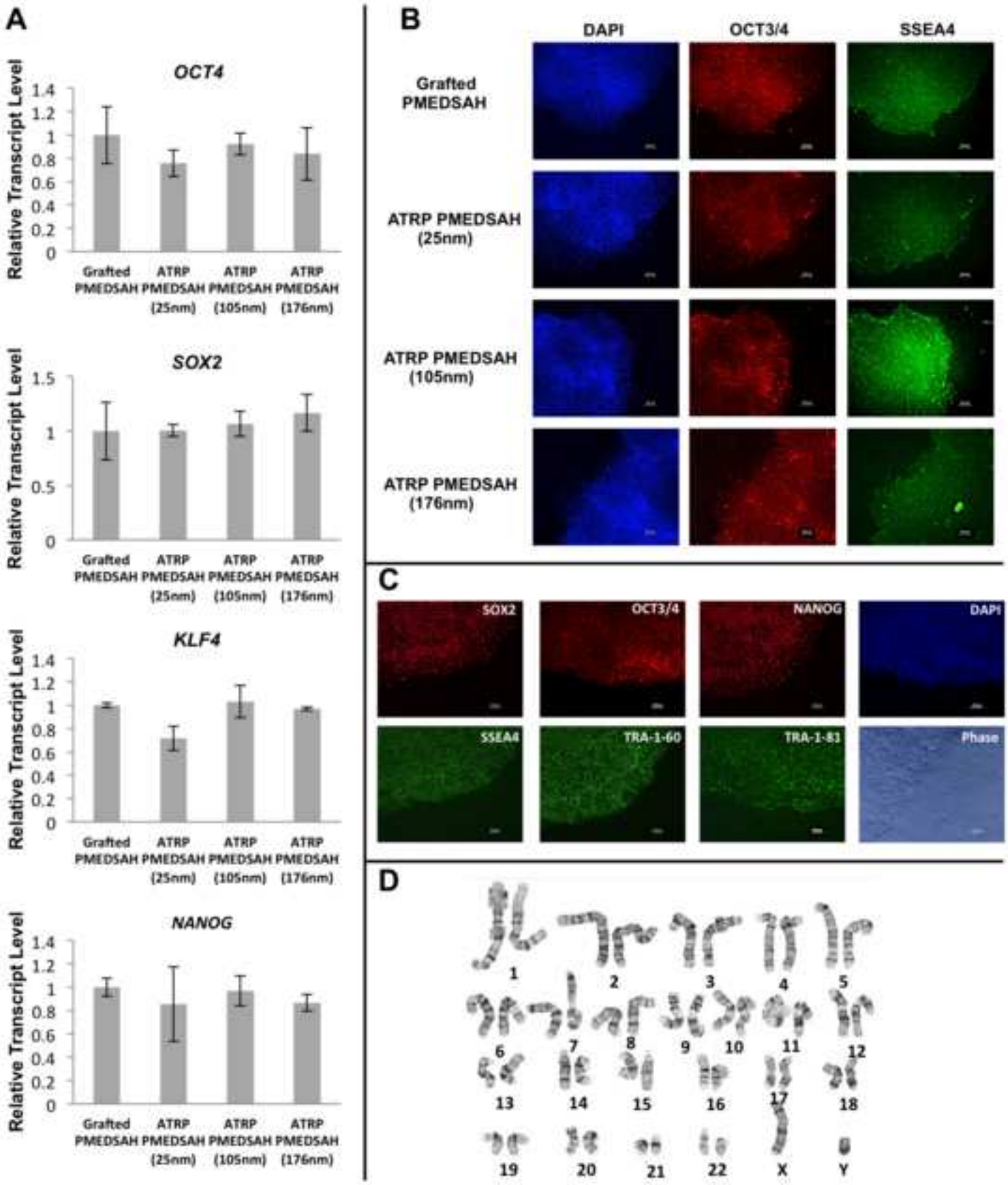
B

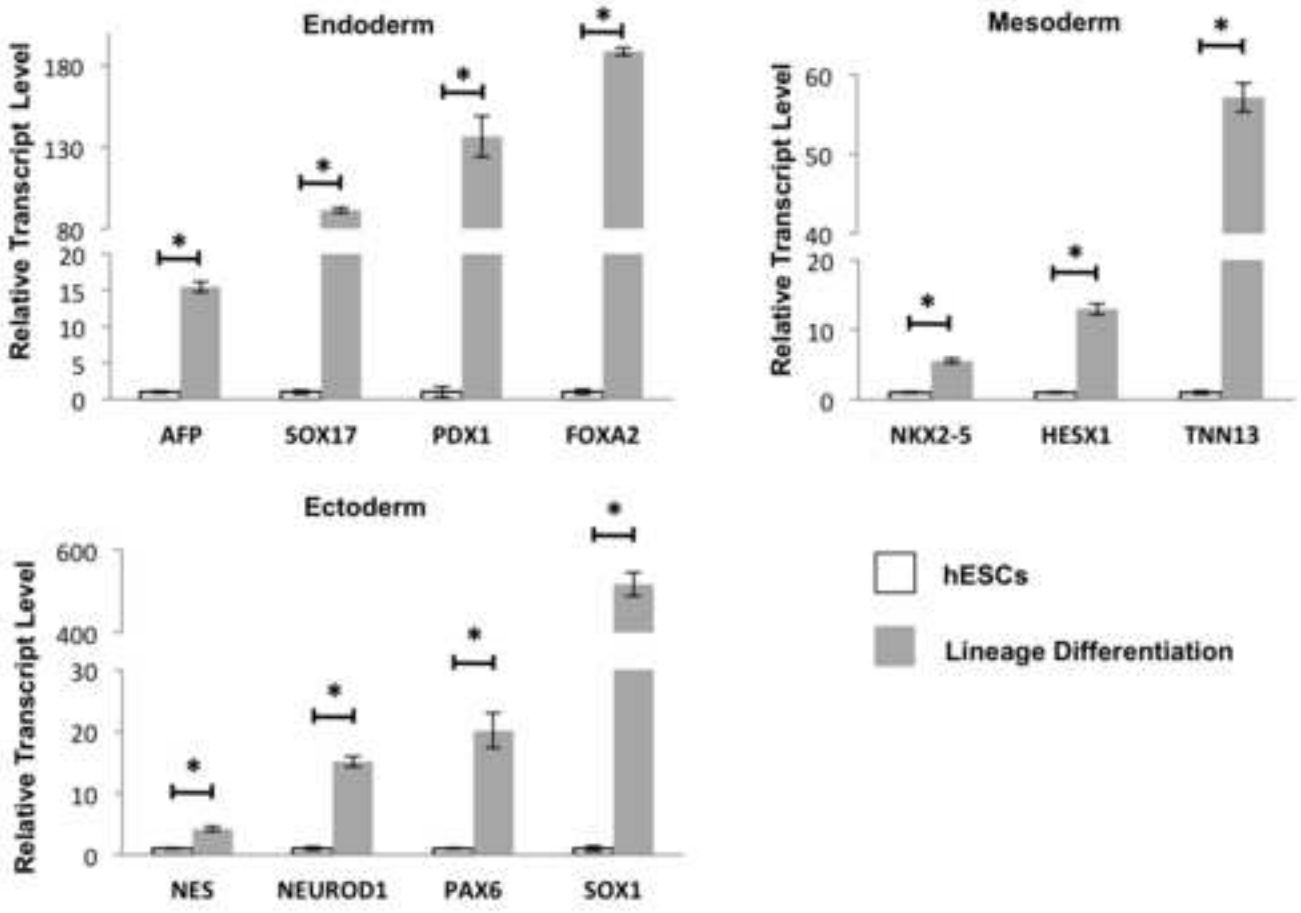

water
Ultraviolet-initiated Polymerization
ATRP
25 nm thick coating
ATRP
105 nm thick coating
ATRP
176 nm thick coating

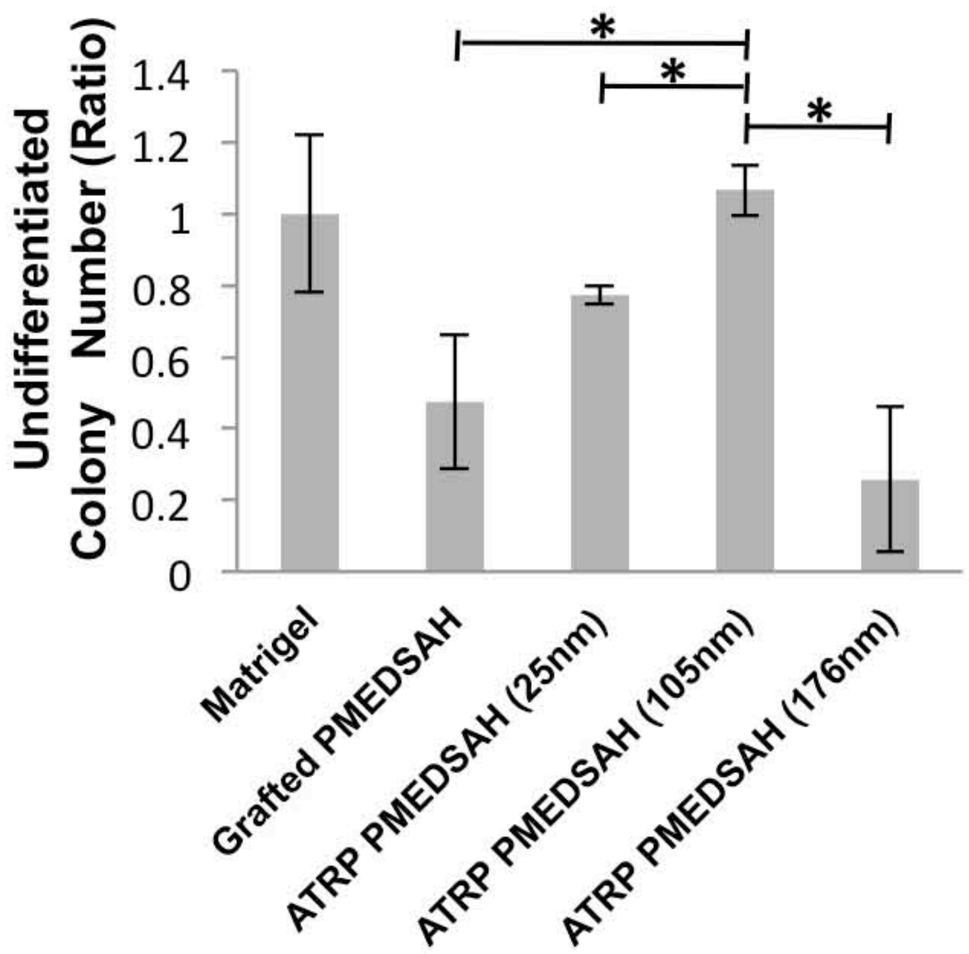
C

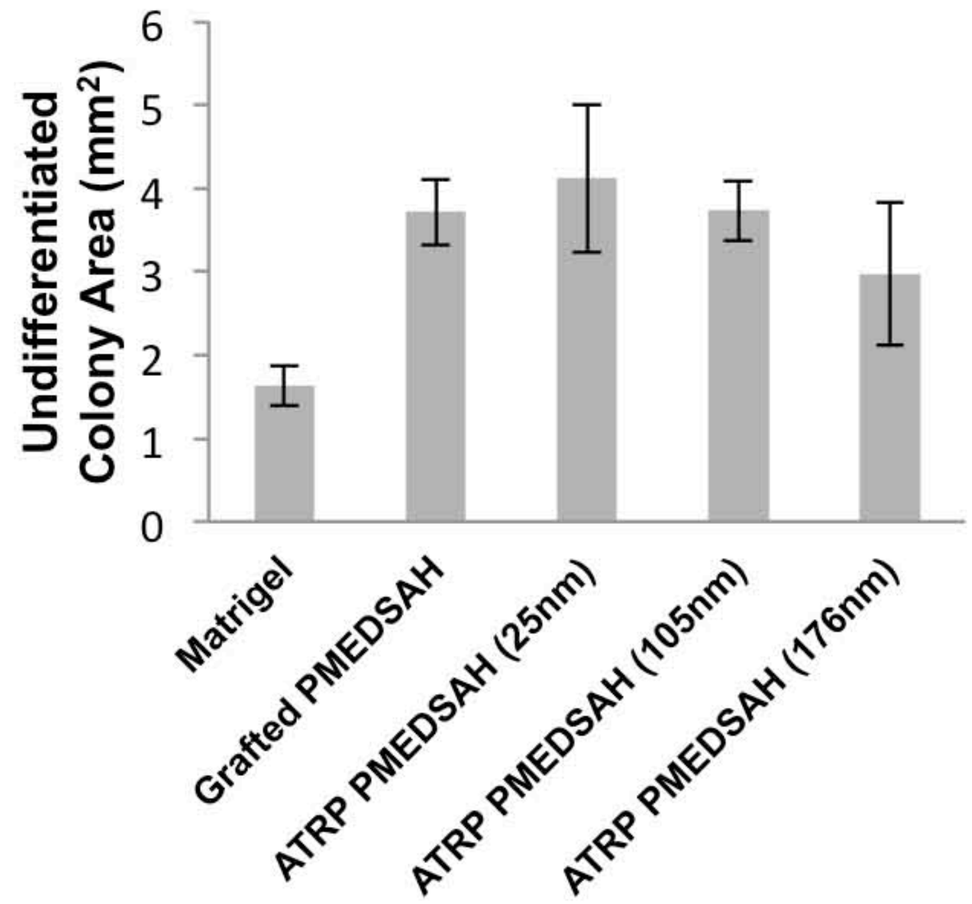

Zeta Potential (mV)
pH
UVO-initated polymerization
ATRP- 25 nm thick coating
ATRP-105 nm thick coating
ATRP- 176 nm thick coating

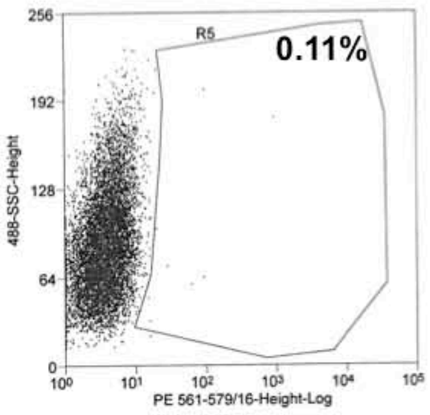
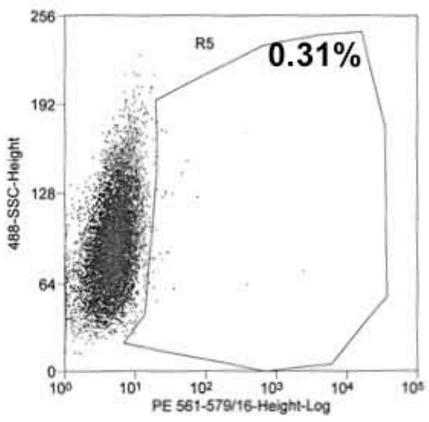
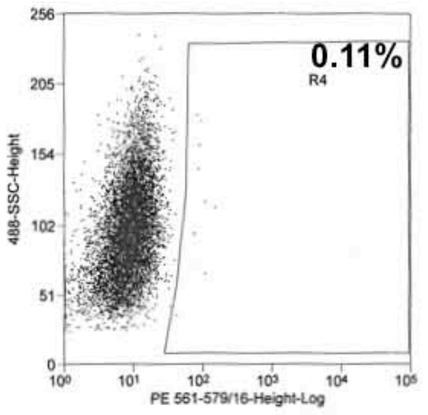
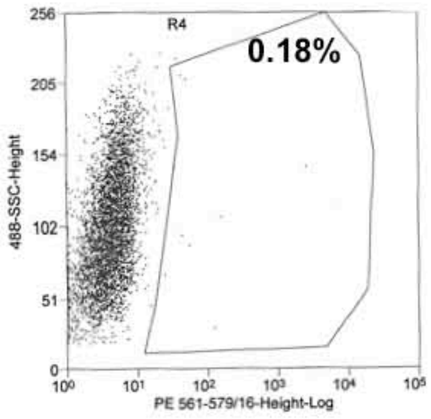
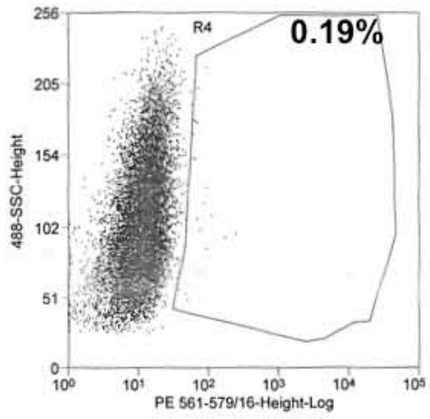
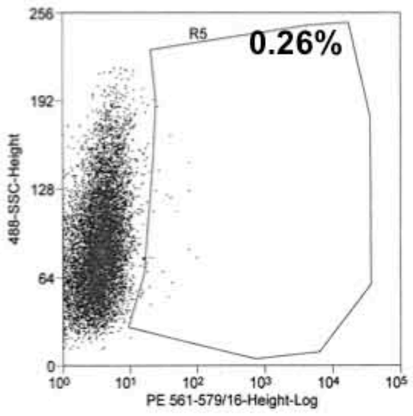
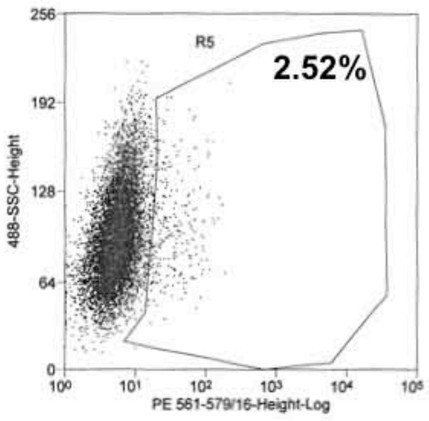
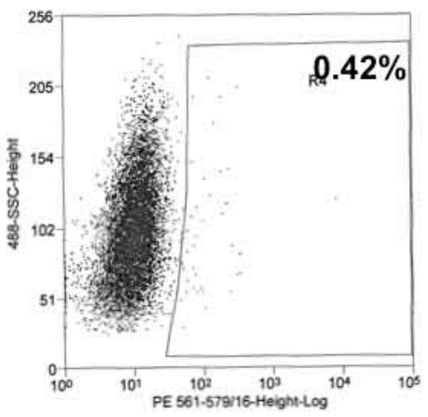
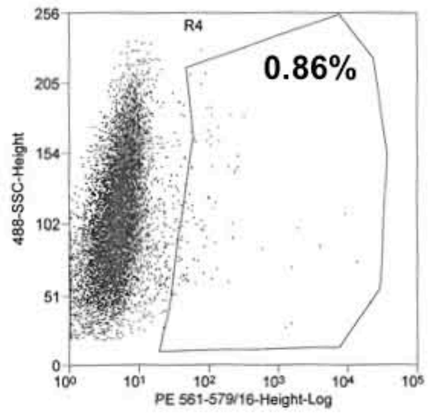
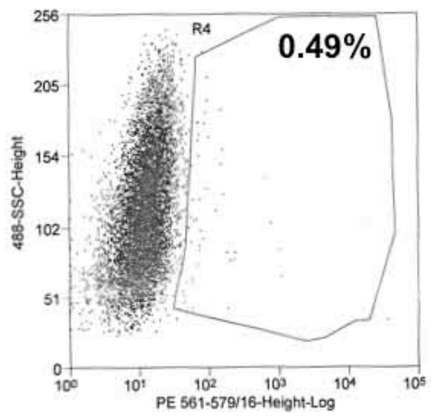
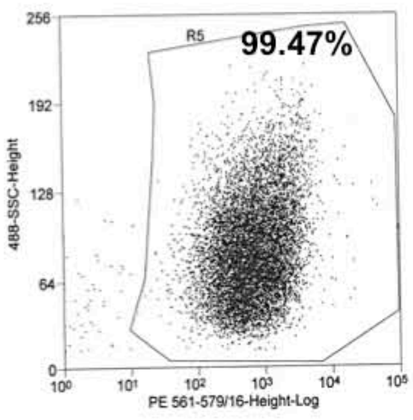
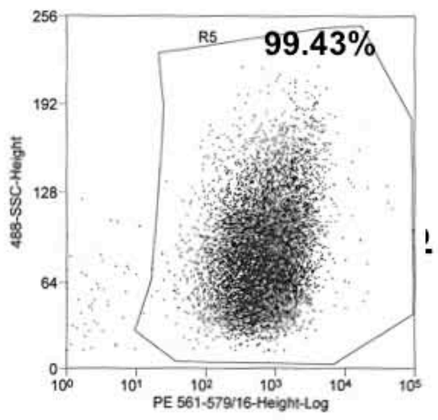
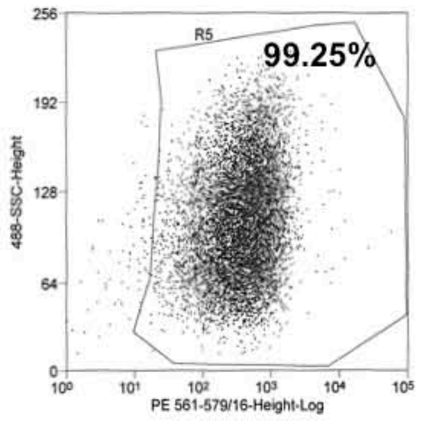
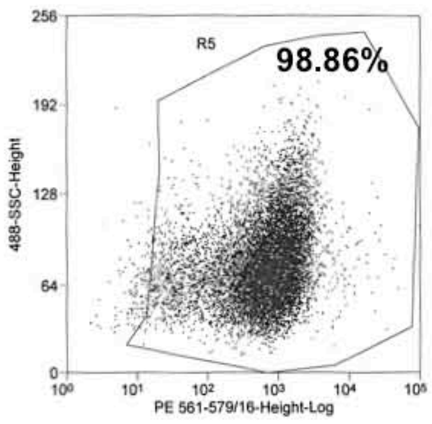
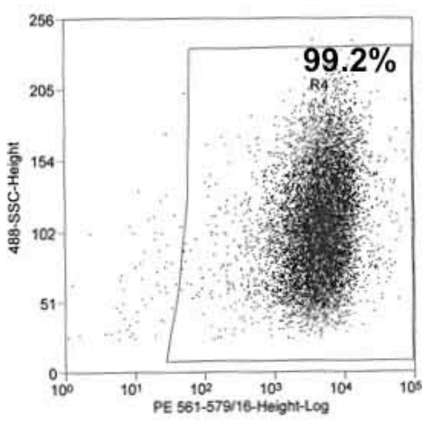
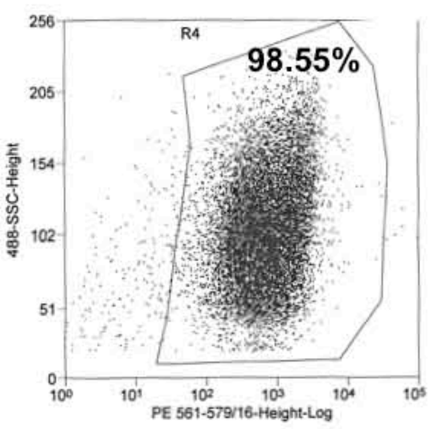
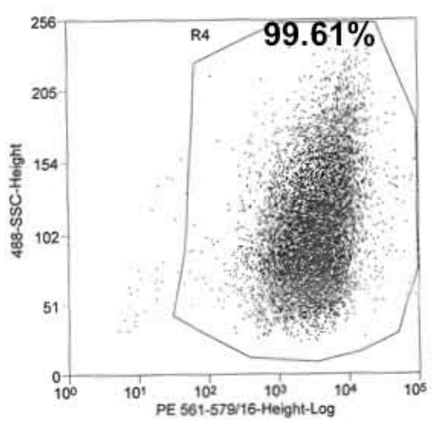
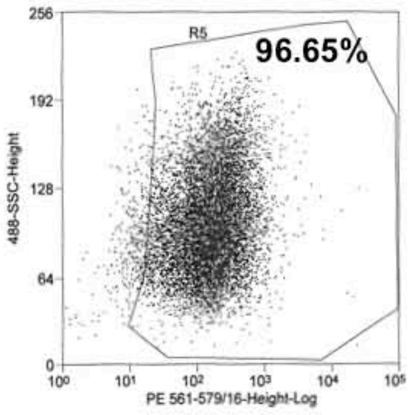
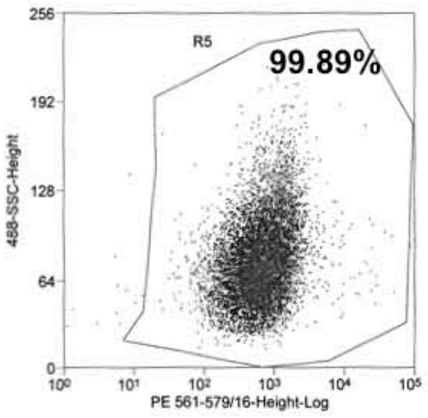
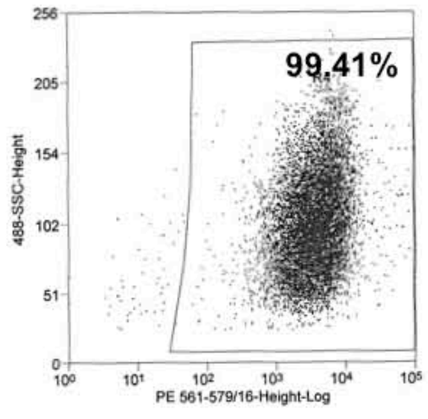
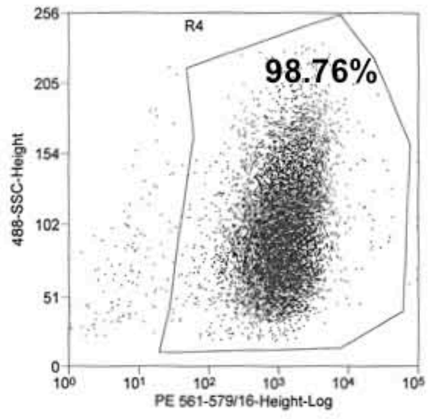
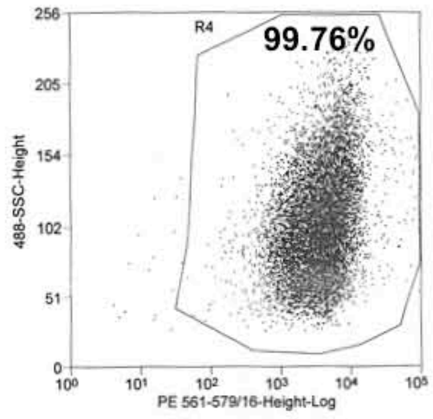
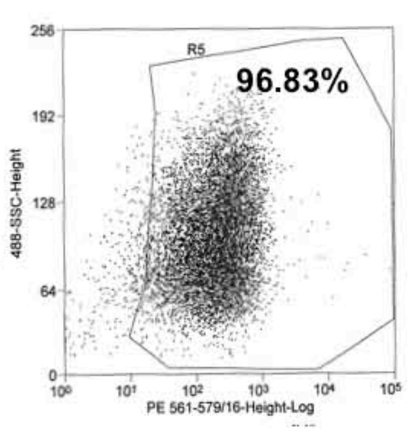
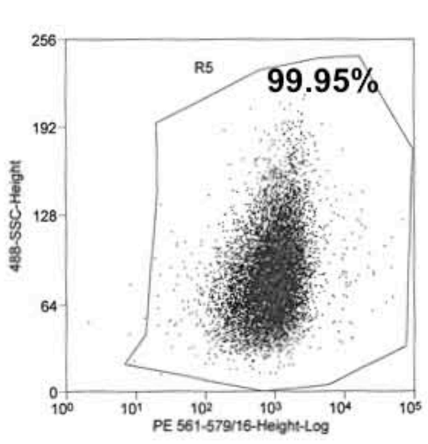
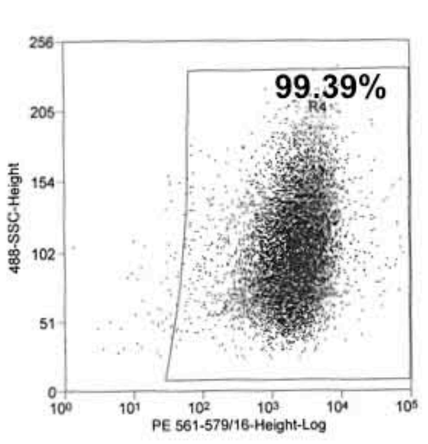
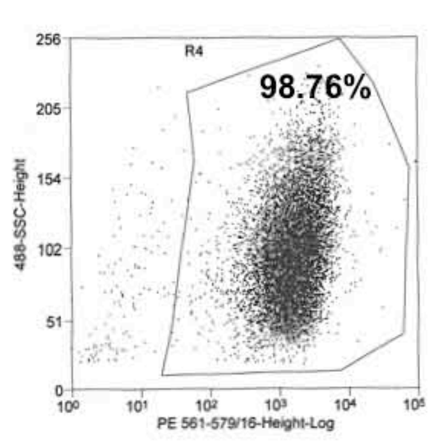
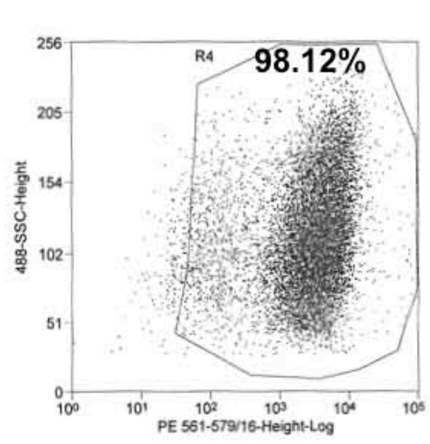

A
Matrigel
Grafted PMEDSAH
ATRP PMEDSAH (25nm)
ATRP PMEDSAH (105nm)
ATRP PMEDSAH (176nm)
B
Undifferentiated Colony Number (Ratio)
Undifferentiated Colony Area (mm²)
Matrigel
Grafted PMEDSAH
ATRP PMEDSAH (25nm)
ATRP PMEDSAH (105nm)
ATRP PMEDSAH (176nm)
C
Week 1
Week 5
Total Cell Number (Ratio)


A
OCT4
SOX2
KLF4
NANOG
Relative Transcript Level
Grafted PMEDSAH
ATRP PMEDSAH (25nm)
ATRP PMEDSAH (105nm)
ATRP PMEDSAH (176nm)
B
DAPI
OCT3/4
SSEA4
Grafted PMEDSAH
ATRP PMEDSAH (25nm)
ATRP PMEDSAH (105nm)
ATRP PMEDSAH (176nm)
C
SOX2
OCT3/4
NANOG
DAPI
SSEA4
TRA-1-60
TRA-1-81
Phase
D


Endoderm
Relative Transcript Level
180
130
80
20
15
10
5
0
AFP
SOX17
PDX1
FOXA2
Mesoderm
Relative Transcript Level
60
50
40
20
10
0
NKX2-5
HESX1
TNN13
Ectoderm
Relative Transcript Level
600
400
30
20
10
0
NES
NEUROD1
PAX6
SOX1
hESCs
Lineage Differentiation


Undifferentiated Colony Number (Ratio)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
*
*
Matrigel
Grafted PMEDSAH
ATRP PMEDSAH (25nm)
ATRP PMEDSAH (105nm)
ATRP PMEDSAH (176nm)


Undifferentiated Colony Area (mm²)
6
5
4
3
2
1
0
Matrigel
Grafted PMEDSAH
ATRP PMEDSAH (25nm)
ATRP PMEDSAH (105nm)
ATRP PMEDSAH (176nm)

	Week 1	Week 2	Week 3	Week 4	Week 5
- Control					
PE					
Matrigel					
Grafted PMEDSAH					
ATRP PMEDSAH (25nm)					
ATRP PMEDSAH (105nm)					
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