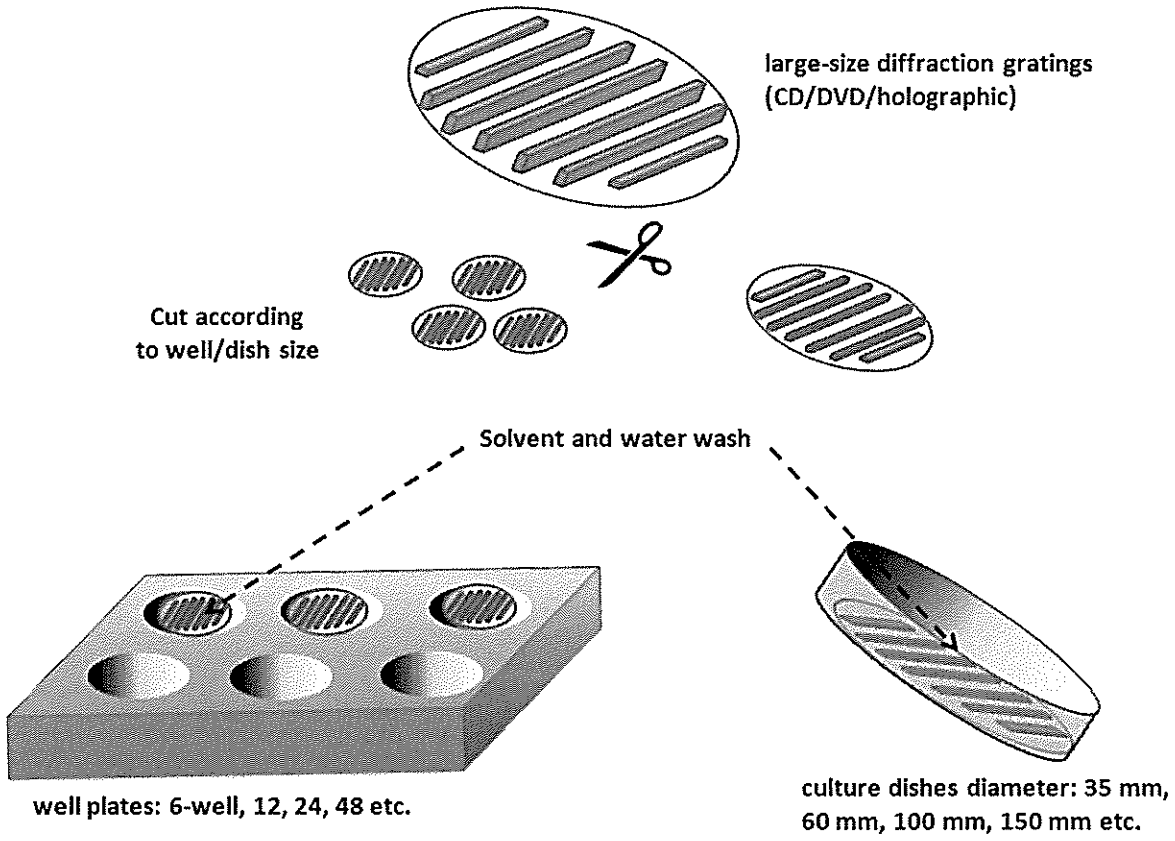
FIG. 1



GRATINGS ON A DISH

FIG. 2

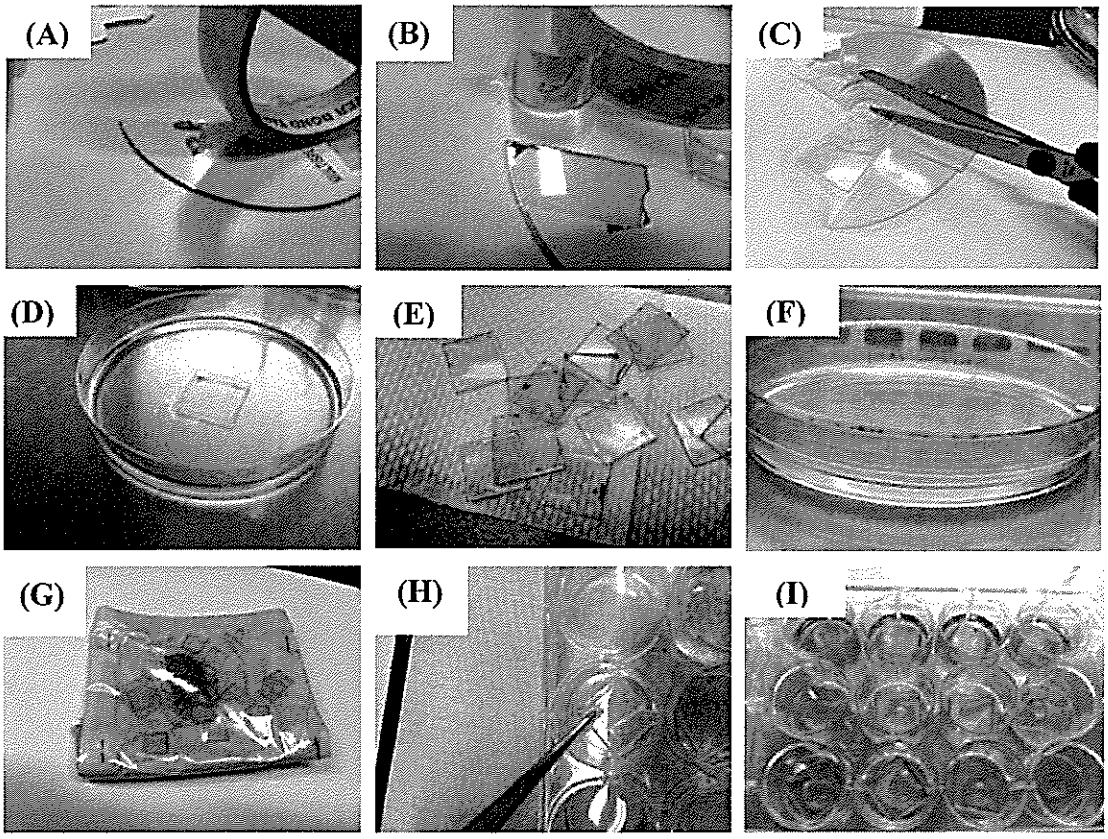

(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)

FIG. 3

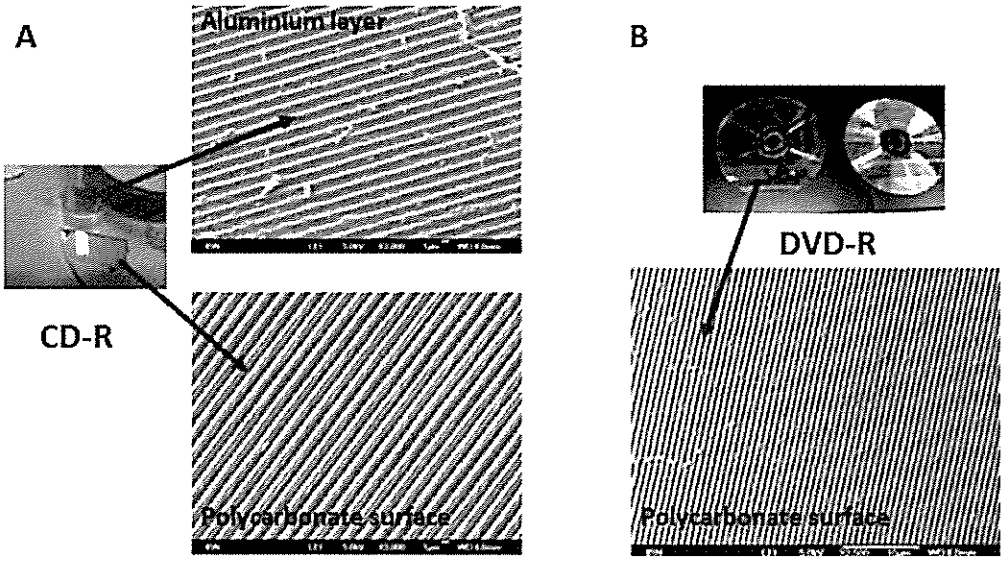

A
Aluminium layer
CD-R
Polycarbonate surface
B
DVD-R
Polycarbonate surface

FIG. 3 (CONT.)

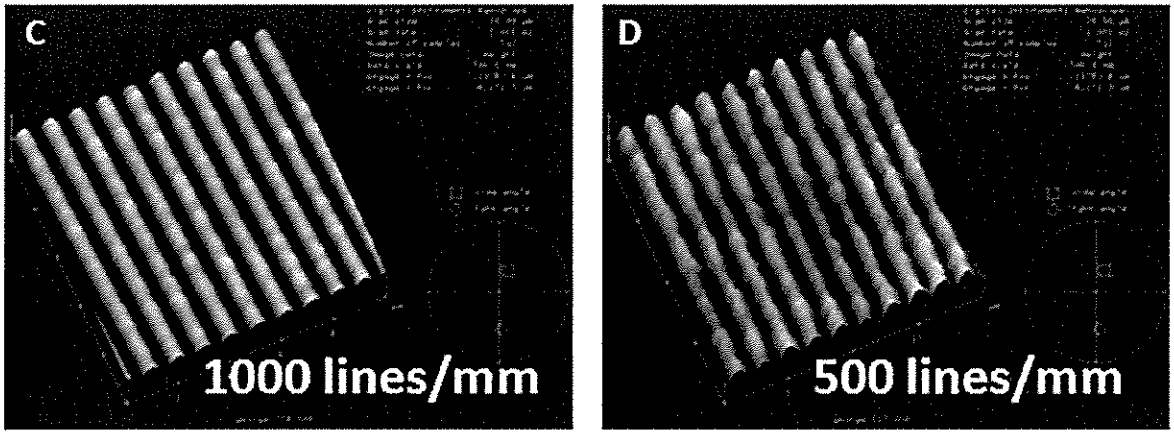


FIG. 4

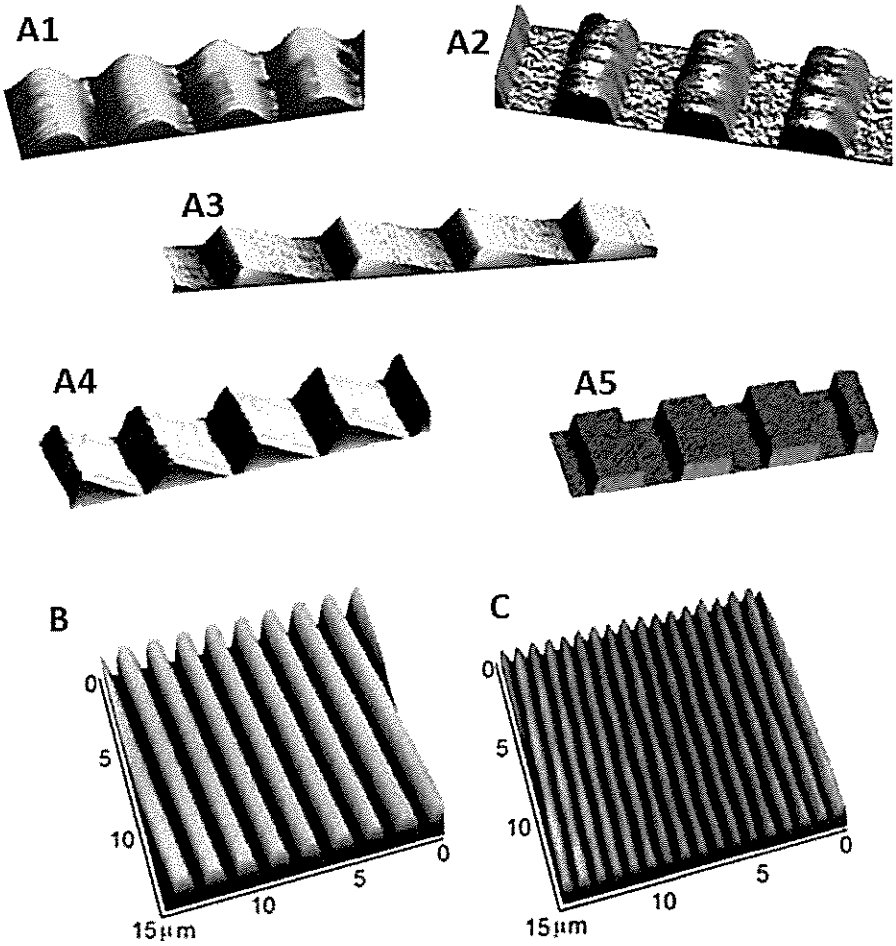


FIG. 5

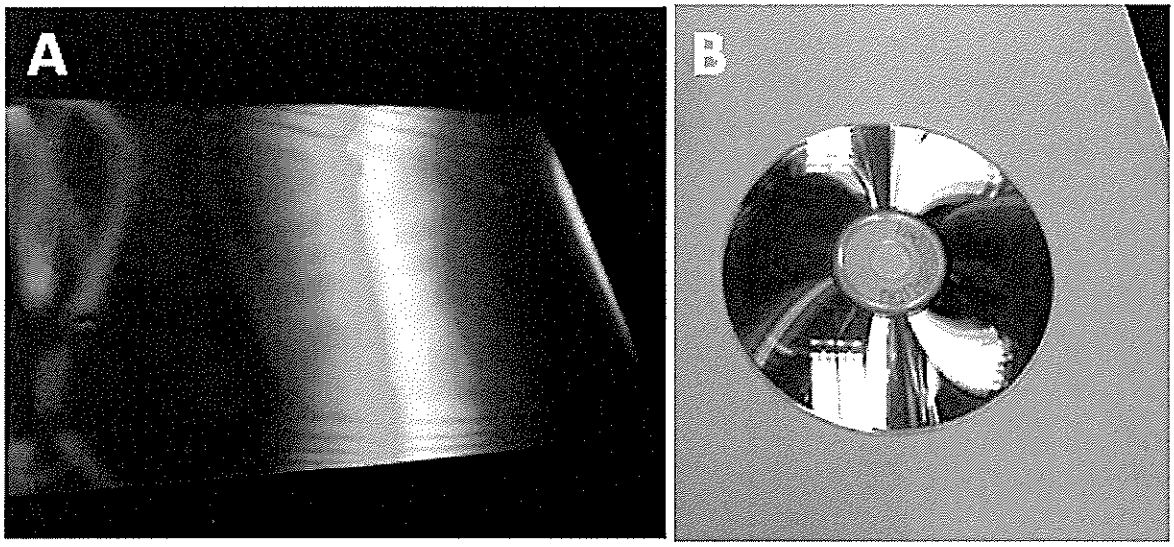


FIG. 6

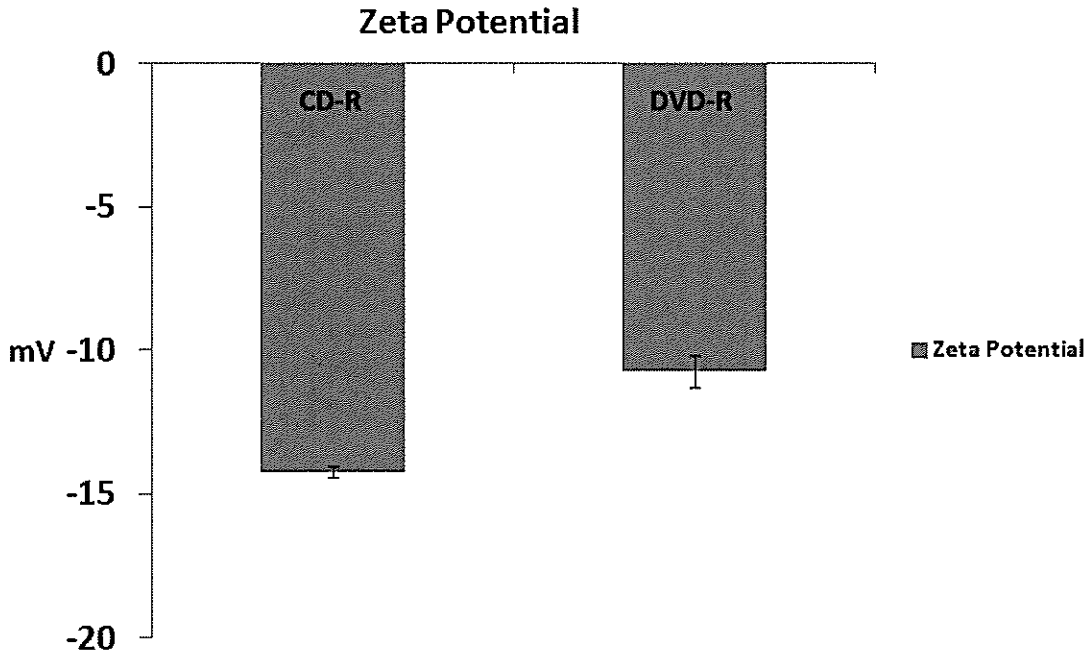


FIG. 7

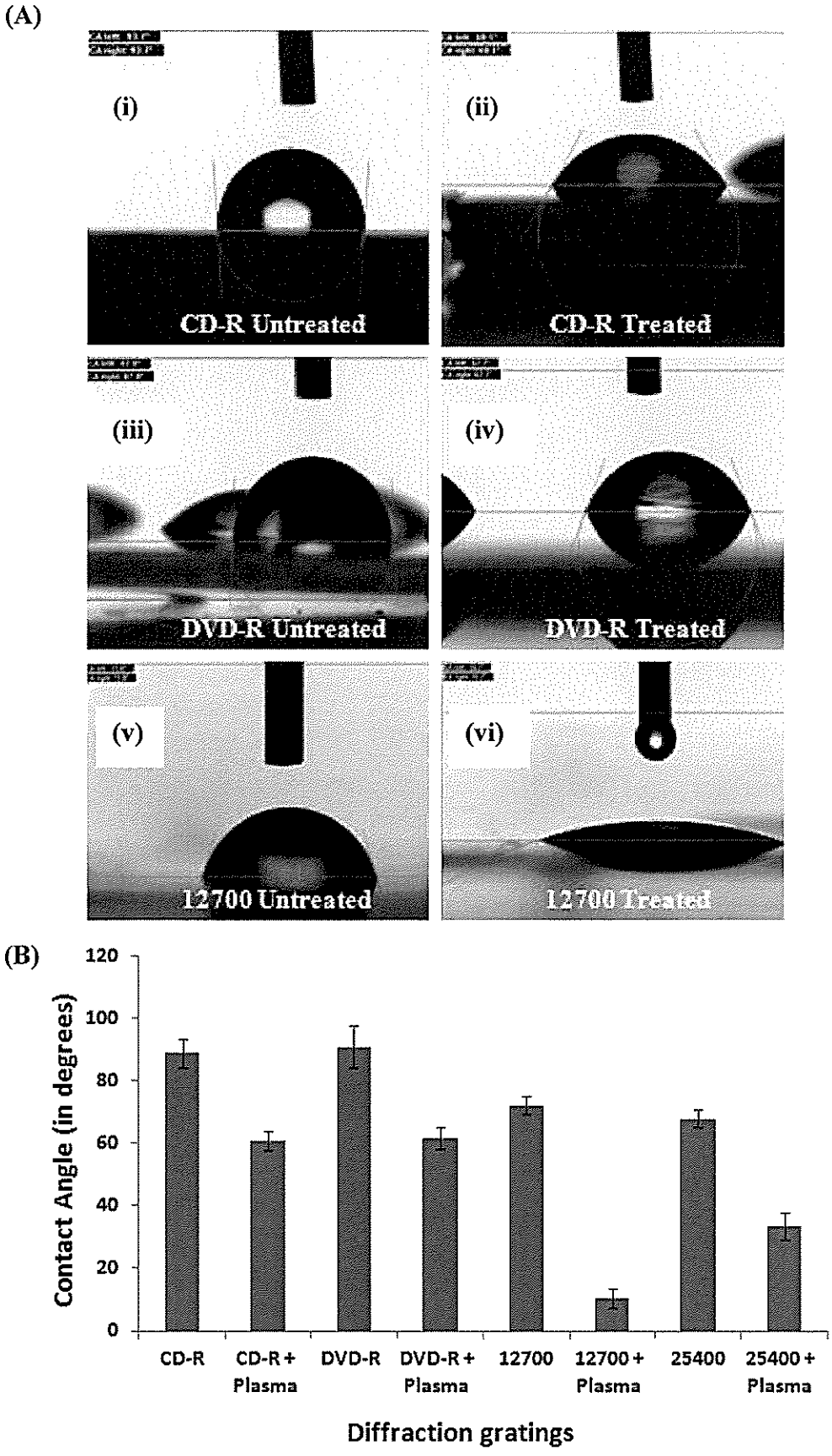

(A)
(i)
CD-R Untreated
(ii)
CD-R Treated
(iii)
DVD-R Untreated
(iv)
DVD-R Treated
(v)
12700 Untreated
(vi)
12700 Treated
(B)
Contact Angle (in degrees)
120
100
80
60
40
20
0
CD-R
CD-R + Plasma
DVD-R
DVD-R + Plasma
12700
12700 + Plasma
25400
25400 + Plasma
Diffraction gratings

FIG. 8

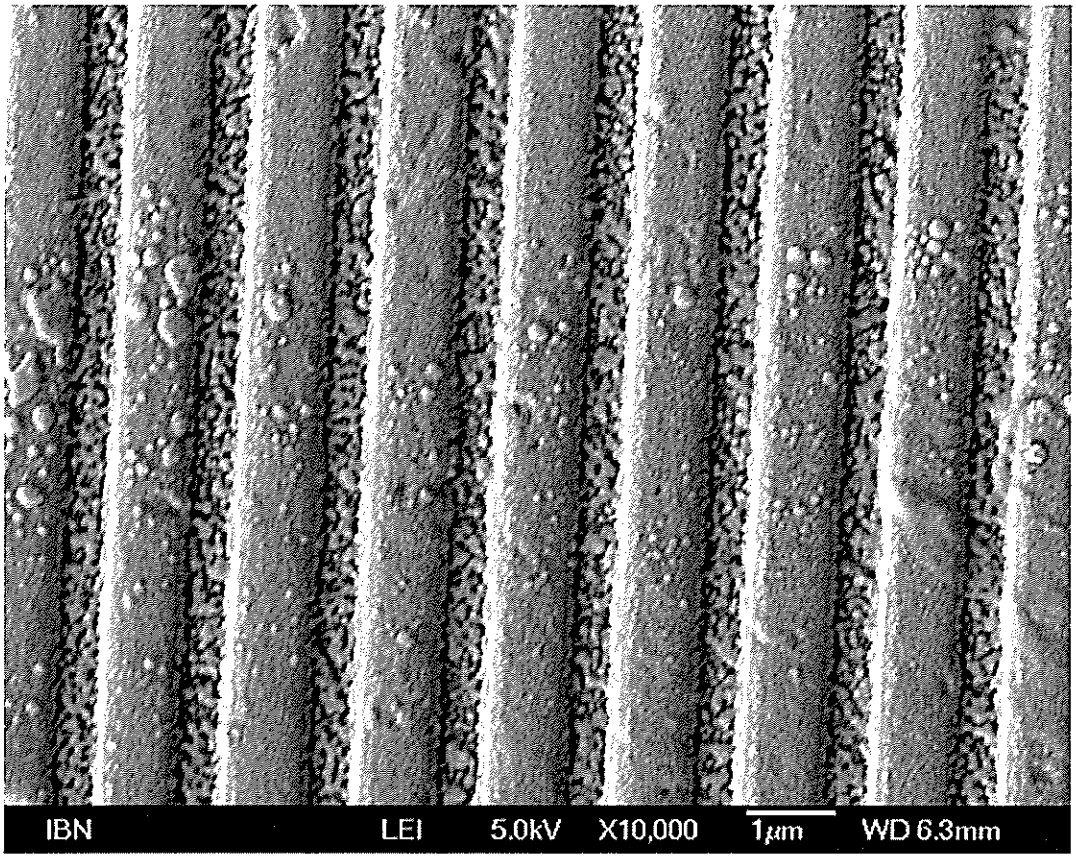

IBN
LEI
5.0kV
X10,000
1μm
WD 6.3mm

FIG. 9

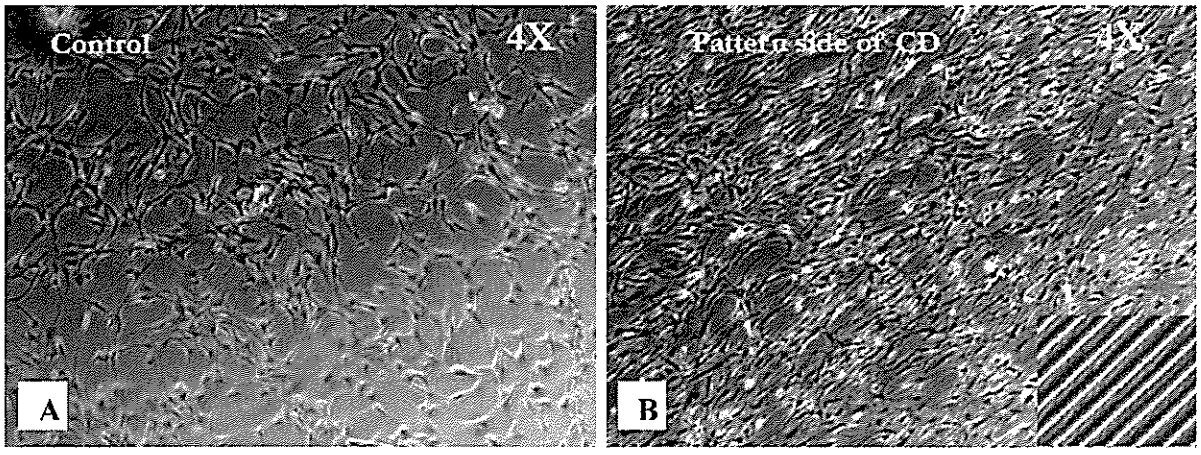

Control
4X
A
Pattern side of CD
4X
B

FIG. 9 (CONT.)

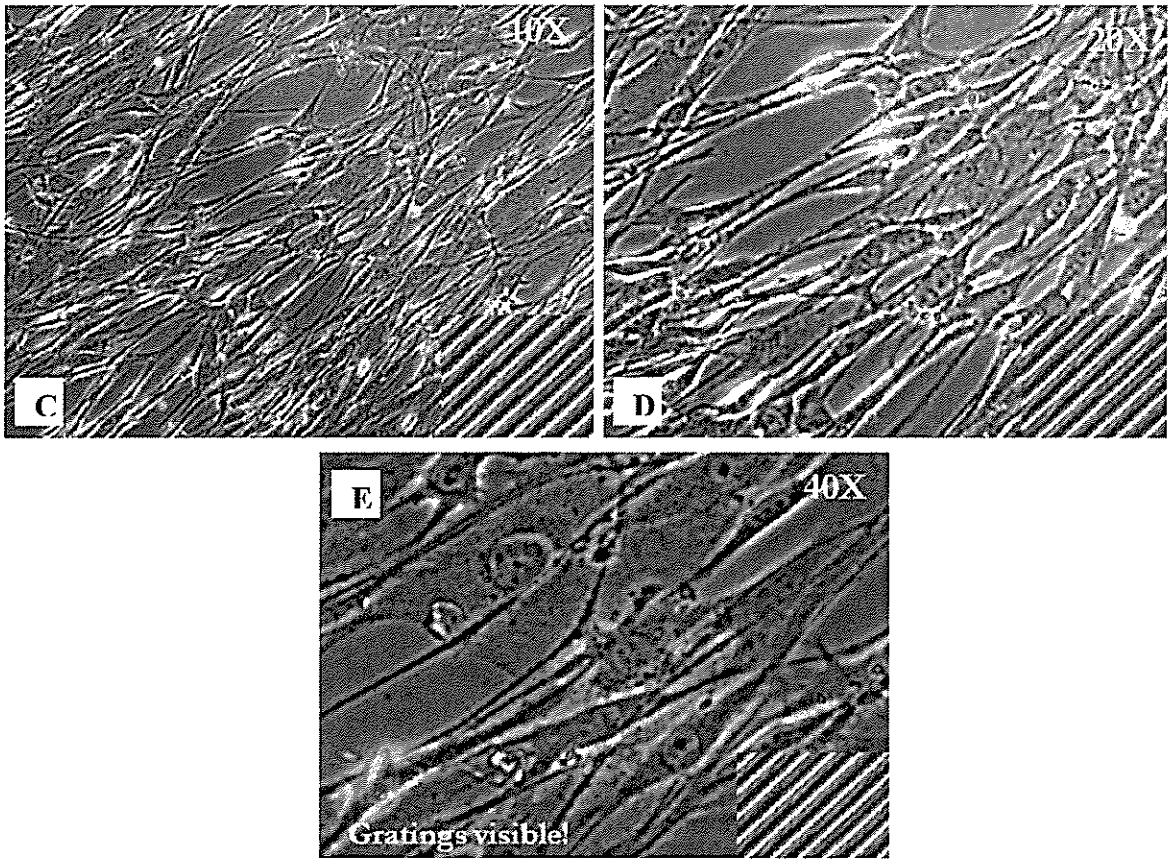


FIG. 10

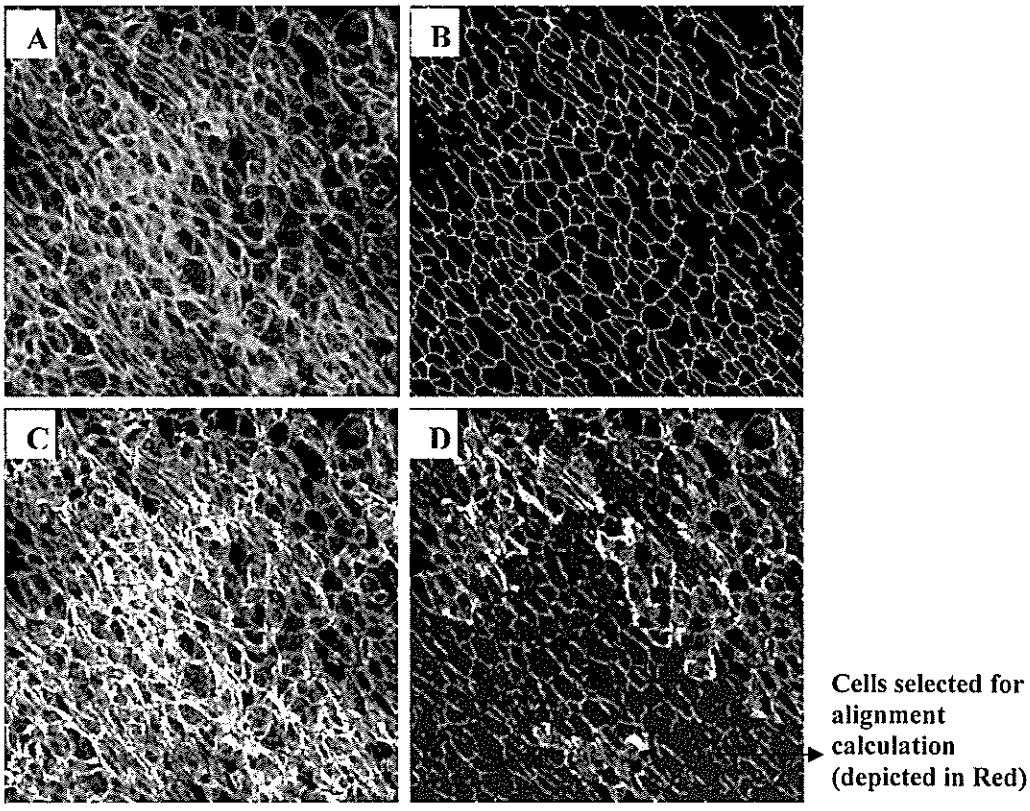


FIG. 10 (CONT.)

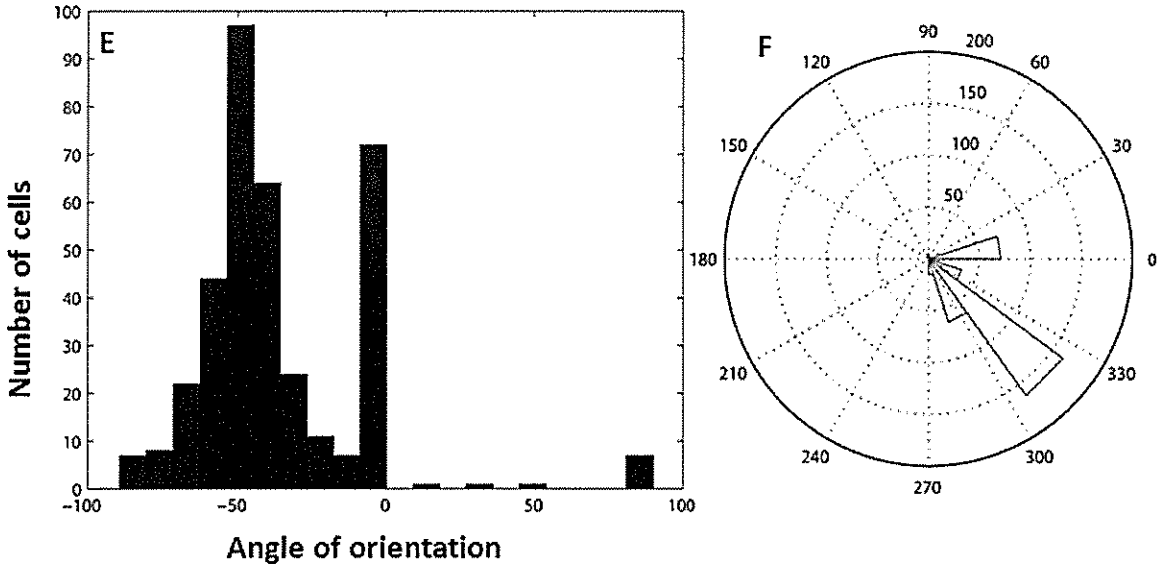


FIG. 11

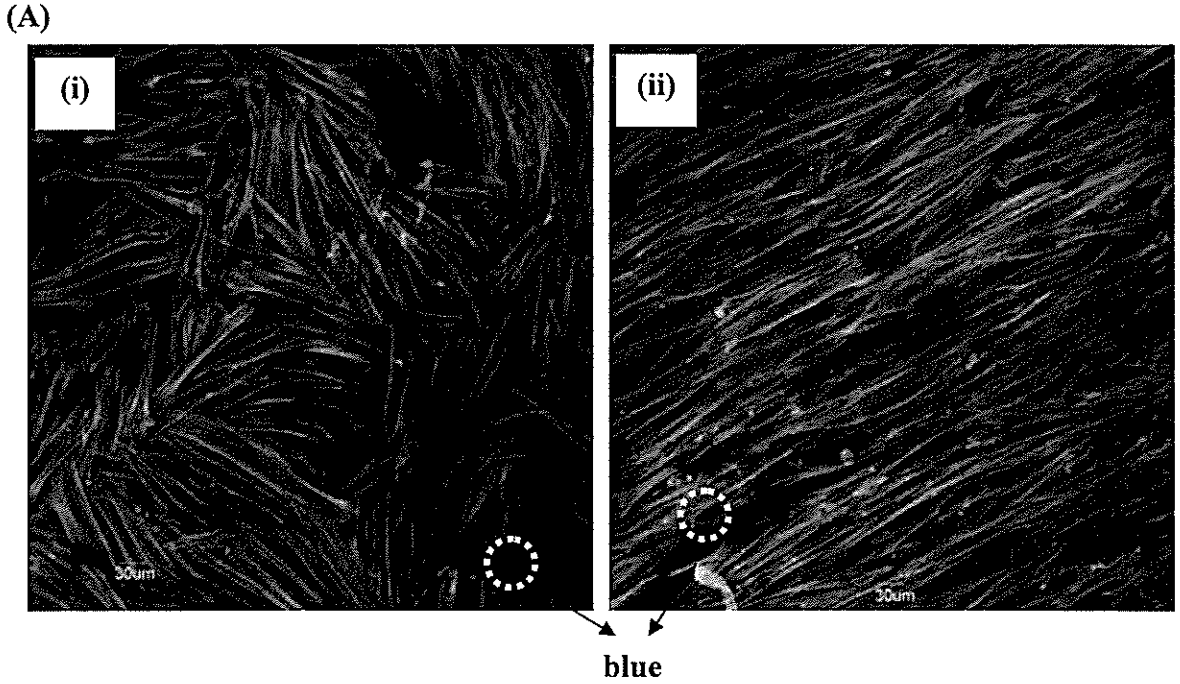


FIG. 11 (CONT.)

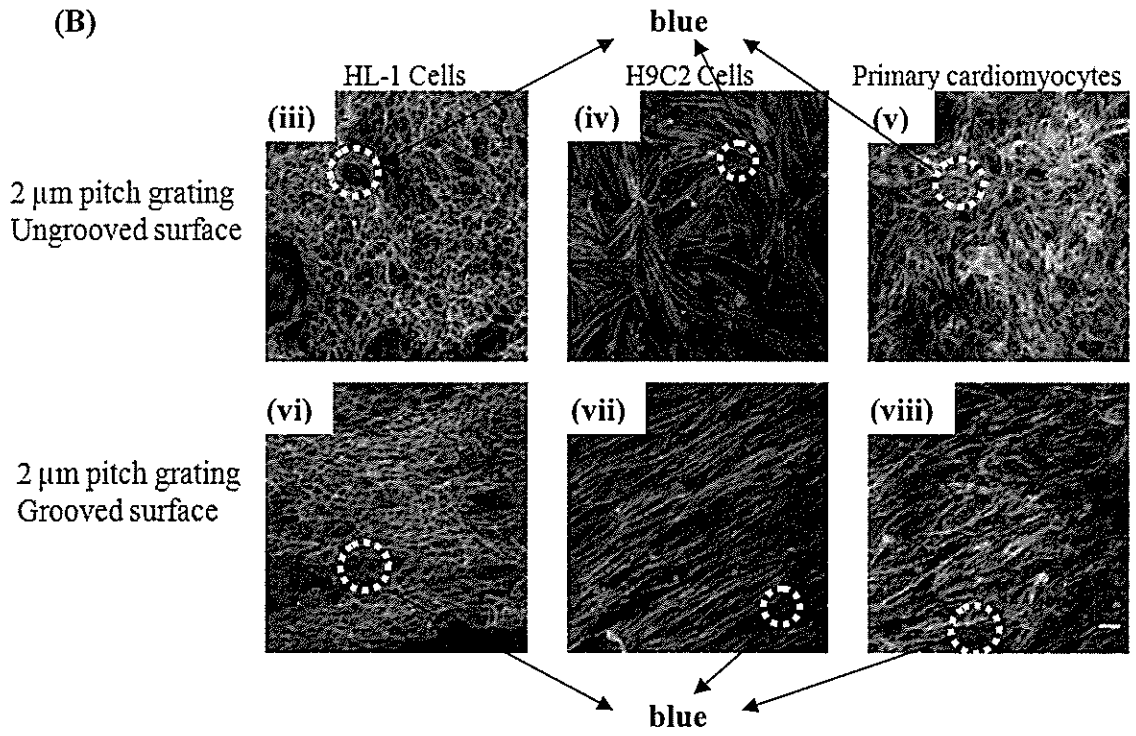


FIG. 12

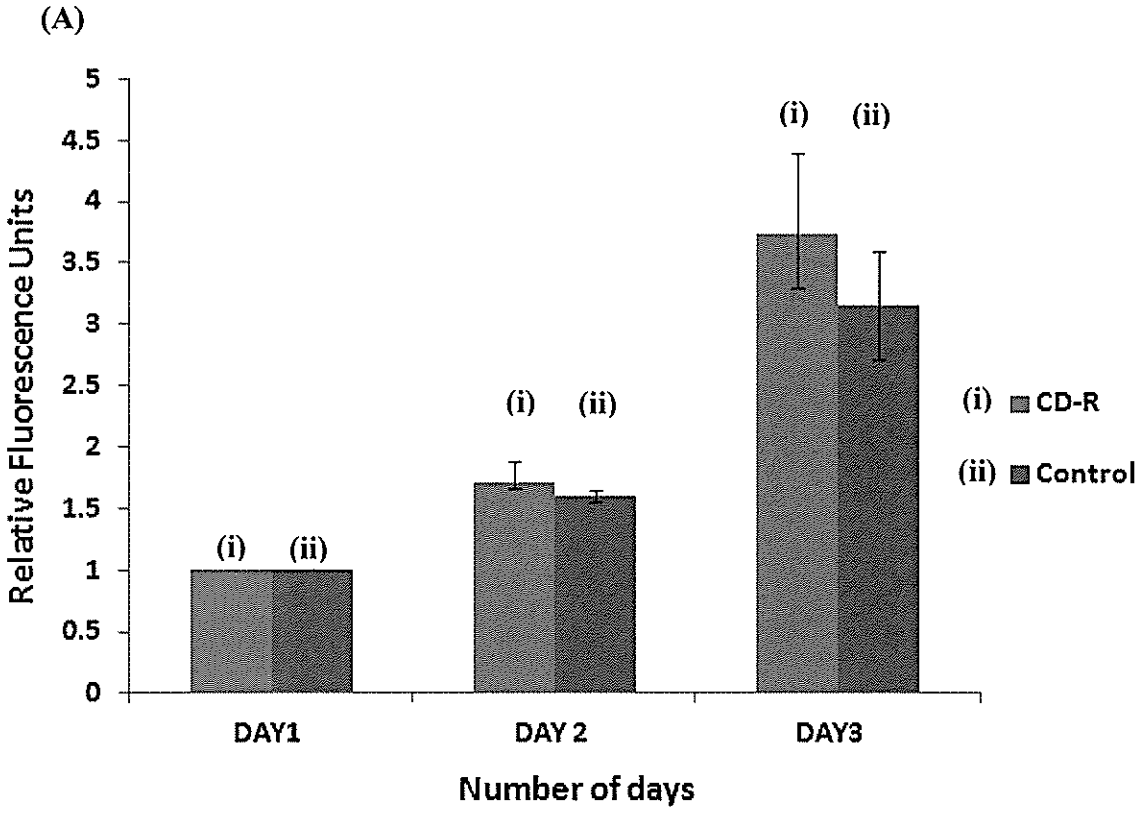


FIG. 12 (CONT.)

(B)

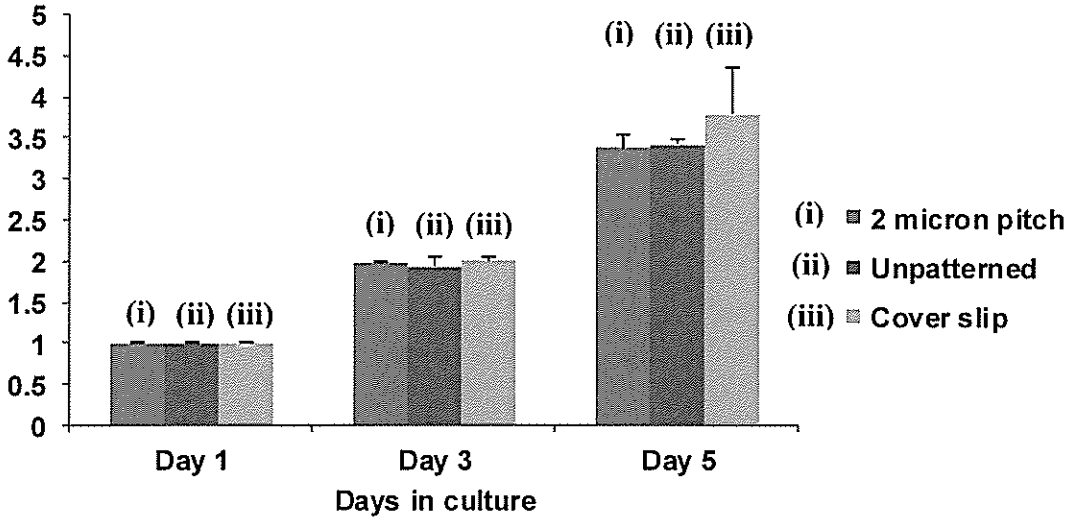
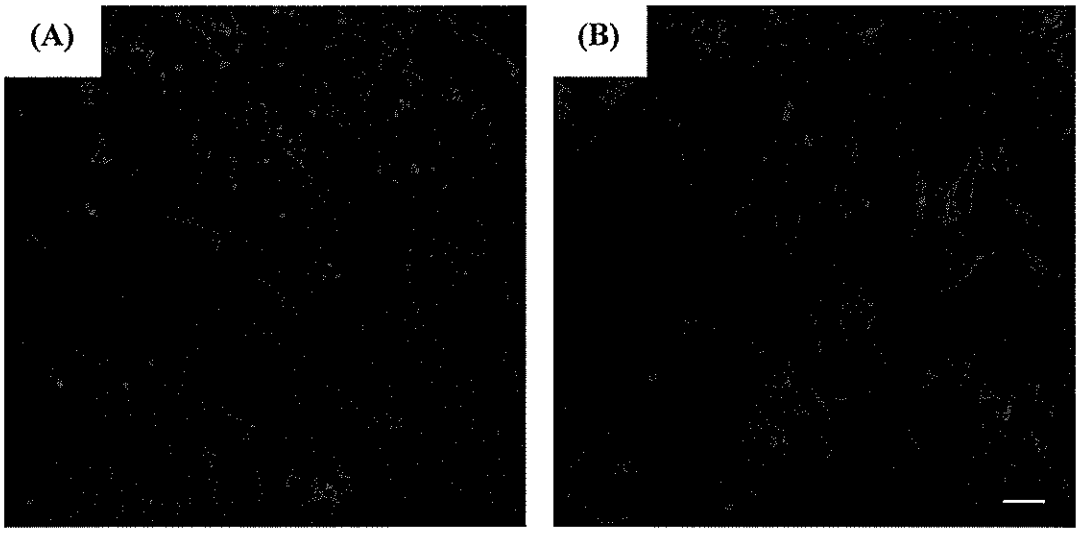


FIG. 13



2 micron pitch

Unpatterned substrate

FIG. 14

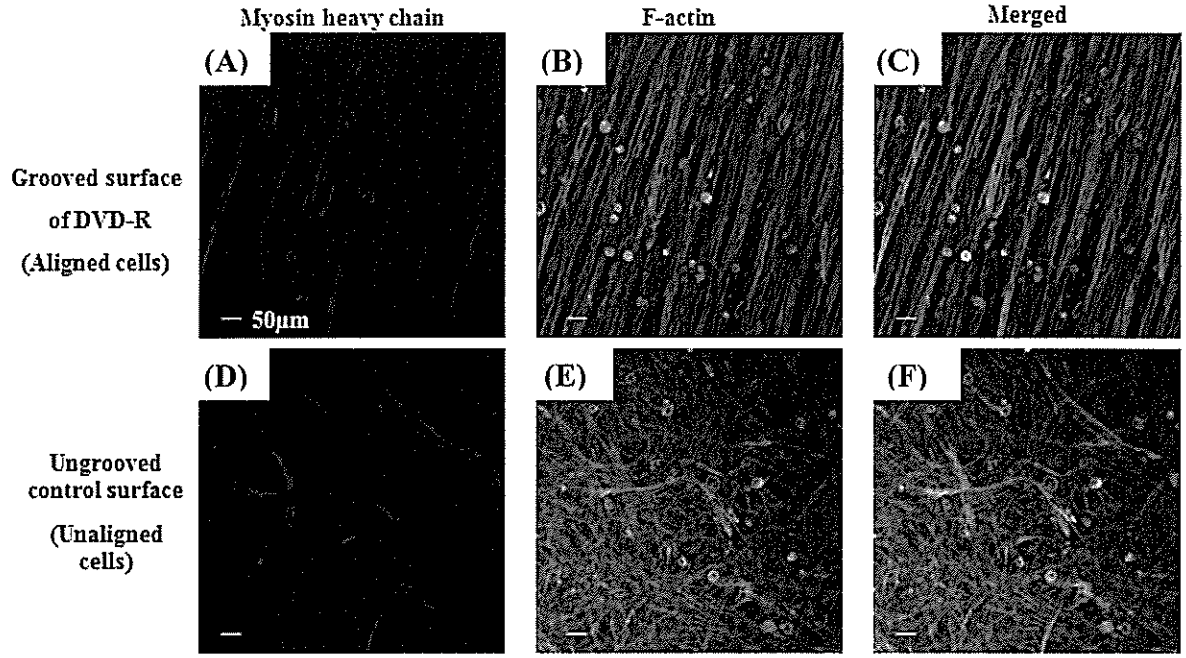

Myosin heavy chain
F-actin
Merged
(A)
(B)
(C)
Grooved surface of DVD-R (Aligned cells)
50μm
(D)
(E)
(F)
Ungrooved control surface (Unaligned cells)

FIG. 15

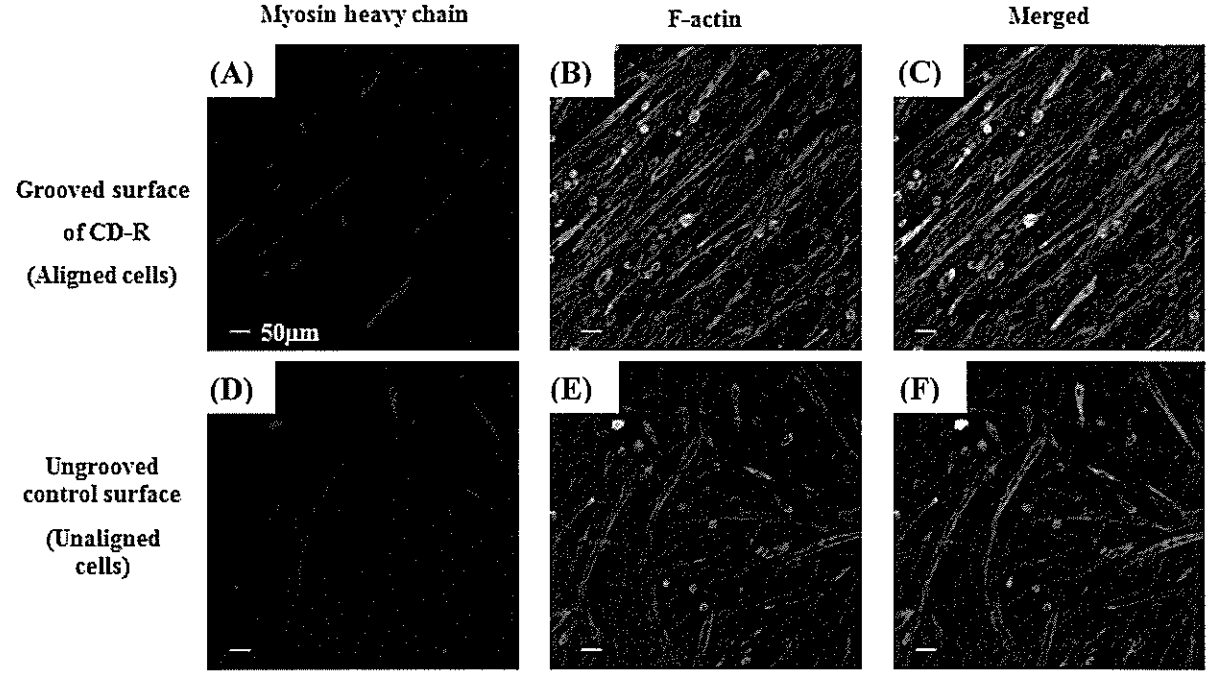

Myosin heavy chain
F-actin
Merged
(A)
(B)
(C)
Grooved surface of CD-R (Aligned cells)
50μm
(D)
(E)
(F)
Ungrooved control surface (Unaligned cells)

FIG. 16

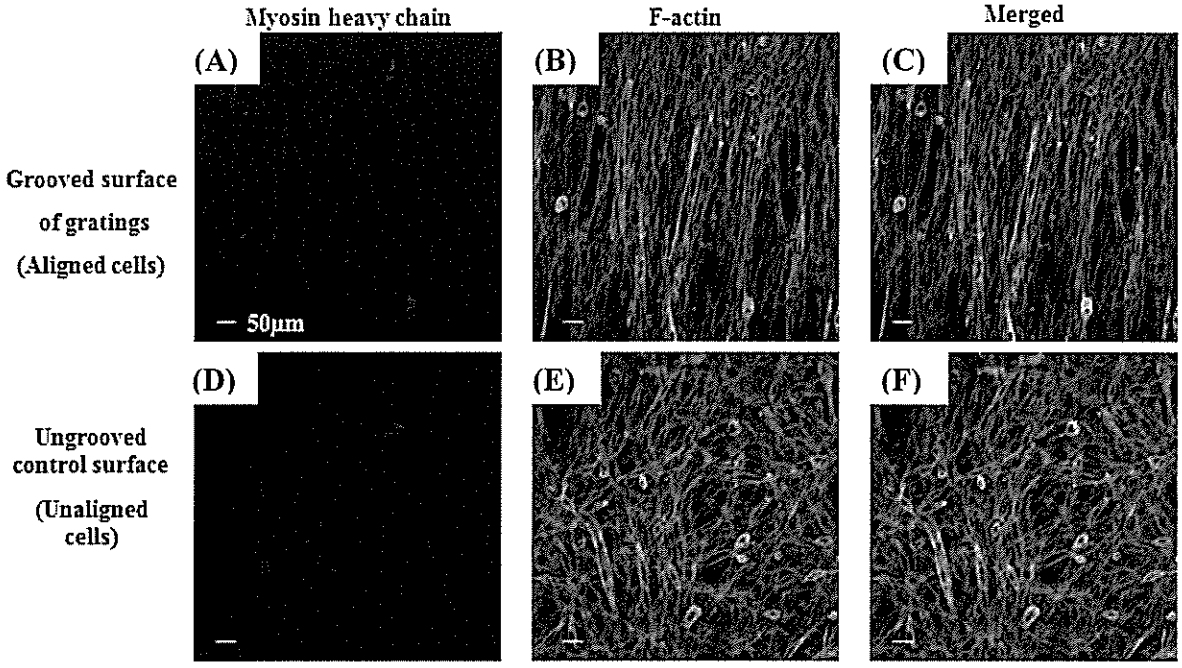

Myosin heavy chain
F-actin
Merged
(A)
(B)
(C)
Grooved surface
of gratings
(Aligned cells)
— 50μm
(D)
(E)
(F)
Ungrooved
control surface
(Unaligned
cells)

FIG. 17

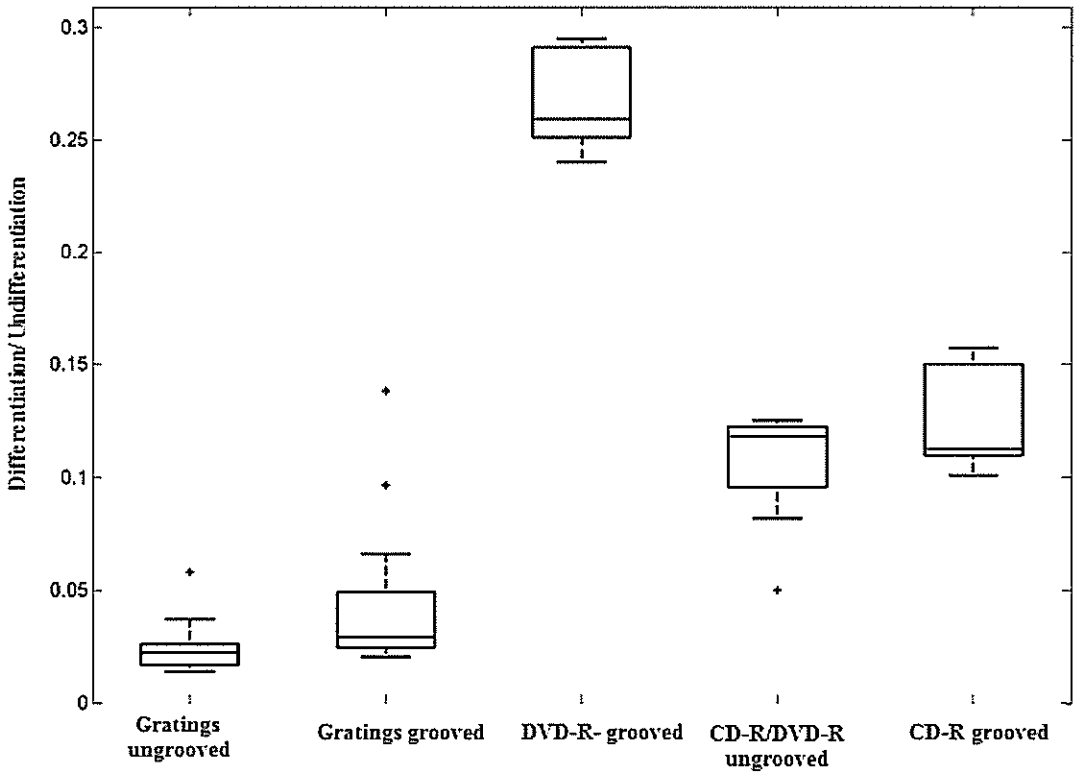

Differentiation/ Undifferentiation
0.3
0.25
0.2
0.15
0.1
0.05
0
Gratings ungrooved
Gratings grooved
DVD-R- grooved
CD-R/DVD-R ungrooved
CD-R grooved

FIG. 18

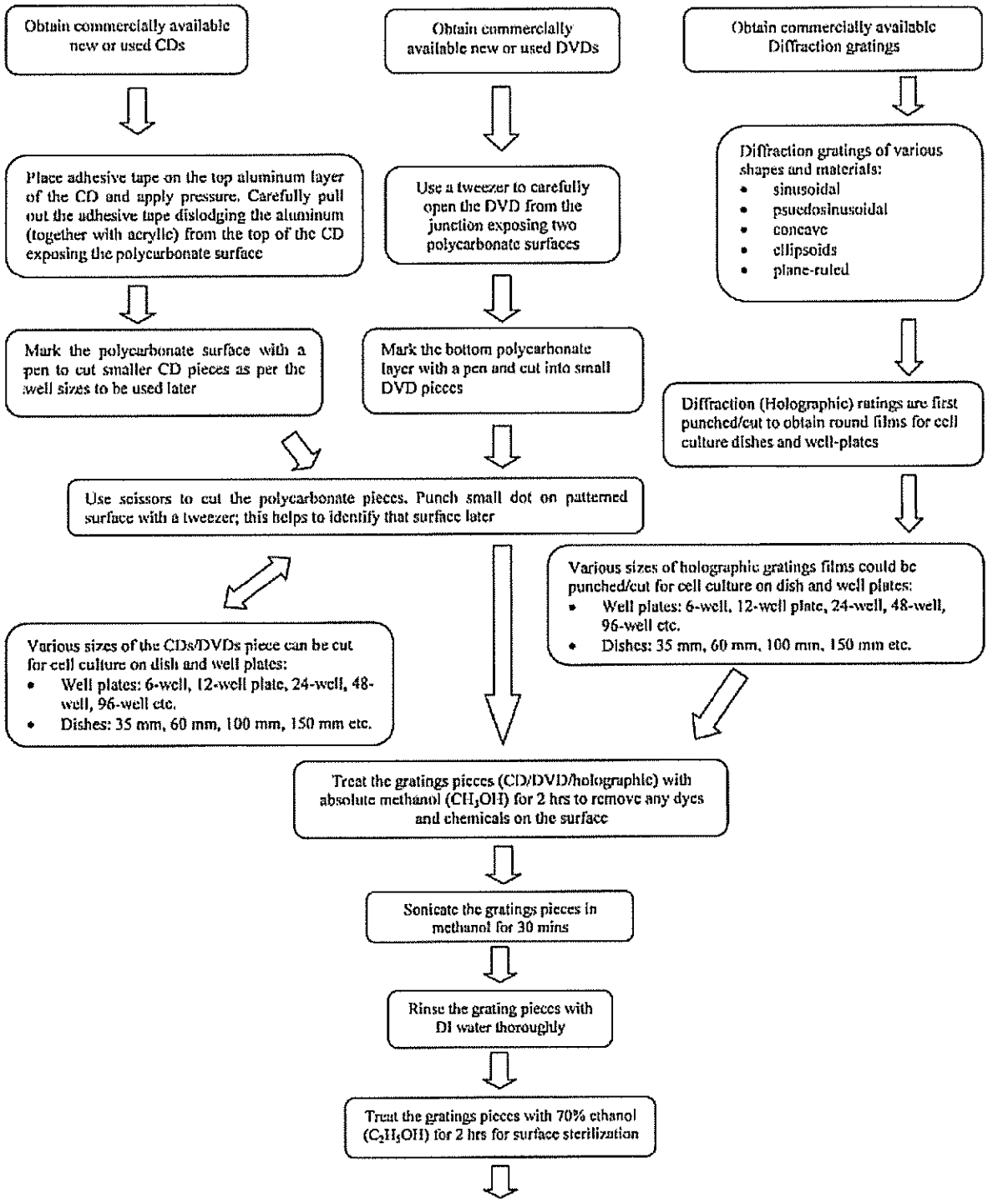
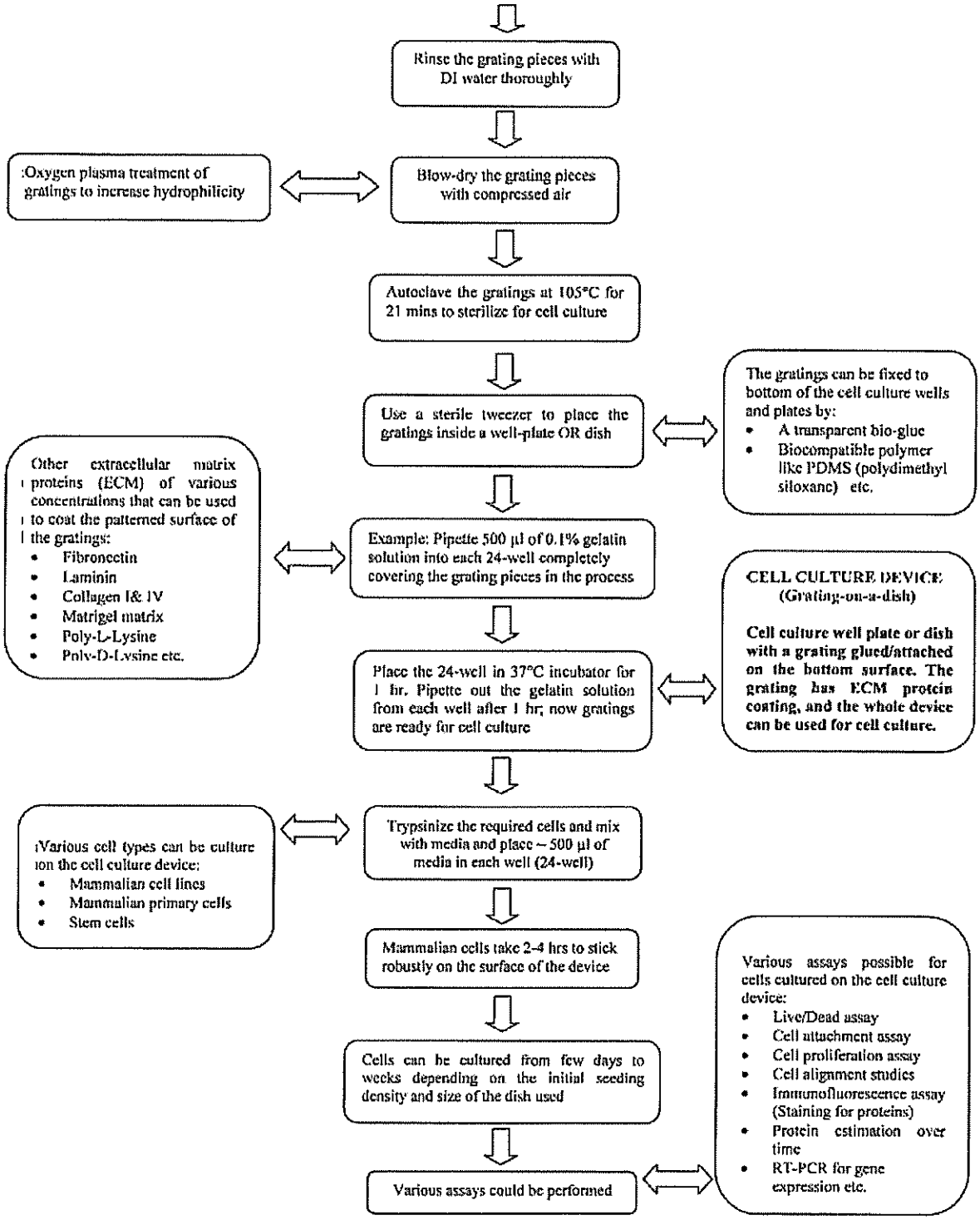

FLOW-CHART OF MAKING "GRATING-ON-A-DISH" AND USAGE
Obtain commercially available new or used CDs
Obtain commercially available new or used DVDs
Obtain commercially available Diffraction gratings
Place adhesive tape on the top aluminum layer of the CD and apply pressure. Carefully pull out the adhesive tape dislodging the aluminum (together with acrylic) from the top of the CD exposing the polycarbonate surface
Use a tweezer to carefully open the DVD from the junction exposing two polycarbonate surfaces
Diffraction gratings of various shapes and materials:
sinusoidal
pseudosinusoidal
concave
ellipsoids
plane-ruled
Mark the polycarbonate surface with a pen to cut smaller CD pieces as per the well sizes to be used later
Mark the bottom polycarbonate layer with a pen and cut into small DVD pieces
Diffraction (Holographic) ratings are first punched/cut to obtain round films for cell culture dishes and well-plates
Use scissors to cut the polycarbonate pieces. Punch small dot on patterned surface with a tweezer; this helps to identify that surface later
Various sizes of holographic gratings films could be punched/cut for cell culture on dish and well plates:
Well plates: 6-well, 12-well plate, 24-well, 48-well, 96-well etc.
Dishes: 35 mm, 60 mm, 100 mm, 150 mm etc.
Various sizes of the CDs/DVDs piece can be cut for cell culture on dish and well plates:
Well plates: 6-well, 12-well plate, 24-well, 48-well, 96-well etc.
Dishes: 35 mm, 60 mm, 100 mm, 150 mm etc.
Treat the gratings pieces (CD/DVD/holographic) with absolute methanol (CH_3OH) for 2 hrs to remove any dyes and chemicals on the surface
Sonicate the gratings pieces in methanol for 30 mins
Rinse the grating pieces with DI water thoroughly
Treat the gratings pieces with 70% ethanol (C_2H_5OH) for 2 hrs for surface sterilization

FIG. 18 (CONT.)


Rinse the grating pieces with DI water thoroughly
Blow-dry the grating pieces with compressed air
:Oxygen plasma treatment of gratings to increase hydrophilicity
Autoclave the gratings at 105°C for 21 mins to sterilize for cell culture
Use a sterile tweezer to place the gratings inside a well-plate OR dish
The gratings can be fixed to bottom of the cell culture wells and plates by:
A transparent bio-glue
Biocompatible polymer like PDMS (polydimethyl siloxane) etc.
Other extracellular matrix proteins (ECM) of various concentrations that can be used to coat the patterned surface of the gratings:
Fibronectin
Laminin
Collagen I& IV
Matrigel matrix
Poly-L-Lysine
Poly-D-Lysine etc.
Example: Pipette 500 µl of 0.1% gelatin solution into each 24-well completely covering the grating pieces in the process
CELL CULTURE DEVICE (Grating-on-a-dish)
Cell culture well plate or dish with a grating glued/attached on the bottom surface. The grating has ECM protein coating, and the whole device can be used for cell culture.
Place the 24-well in 37°C incubator for 1 hr. Pipette out the gelatin solution from each well after 1 hr; now gratings are ready for cell culture
:Various cell types can be culture ion the cell culture device:
Mammalian cell lines
Mammalian primary cells
Stem cells
Trypsinize the required cells and mix with media and place ~ 500 µl of media in each well (24-well)
Mammalian cells take 2-4 hrs to stick robustly on the surface of the device
Cells can be cultured from few days to weeks depending on the initial seeding density and size of the dish used
Various assays could be performed
Various assays possible for cells cultured on the cell culture device:
Live/Dead assay
Cell attachment assay
Cell proliferation assay
Cell alignment studies
Immunofluorescence assay (Staining for proteins)
Protein estimation over time
RT-PCR for gene expression etc.