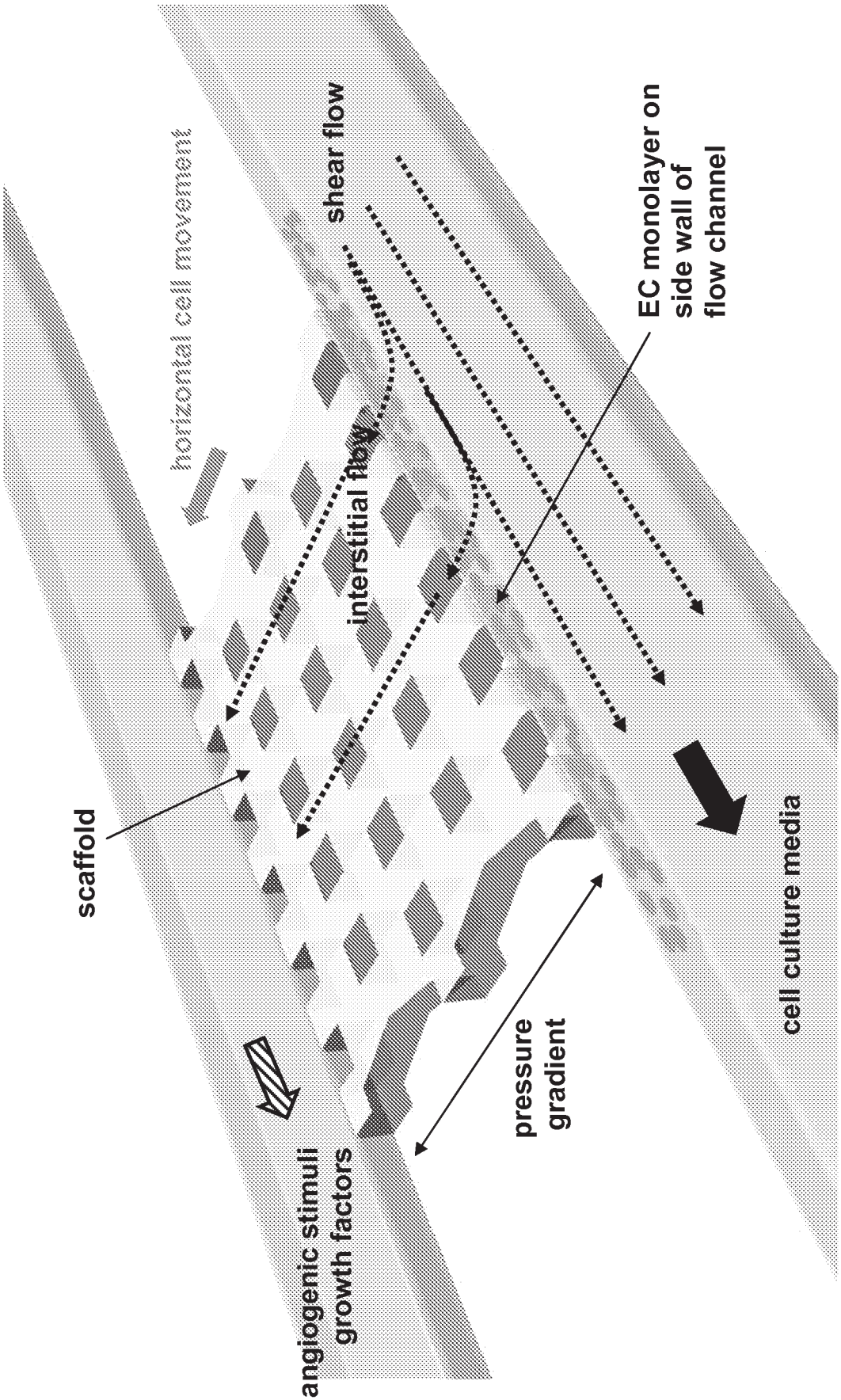

horizontal cell movement
shear flow
interstitial flow
EC monolayer on side wall of flow channel
scaffold
angiogenic stimuli growth factors
pressure gradient
cell culture media

Figure 1

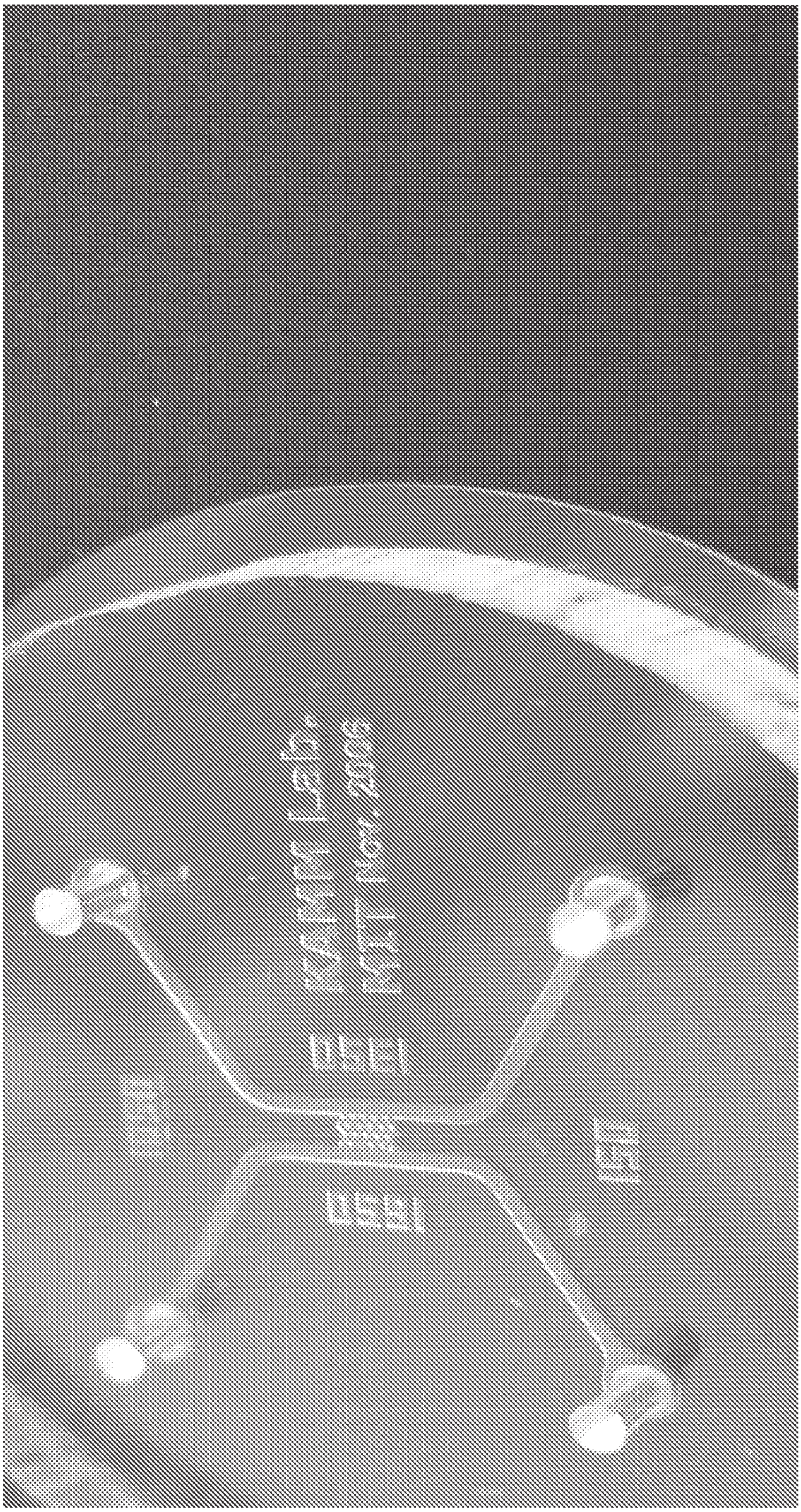


Figure 2

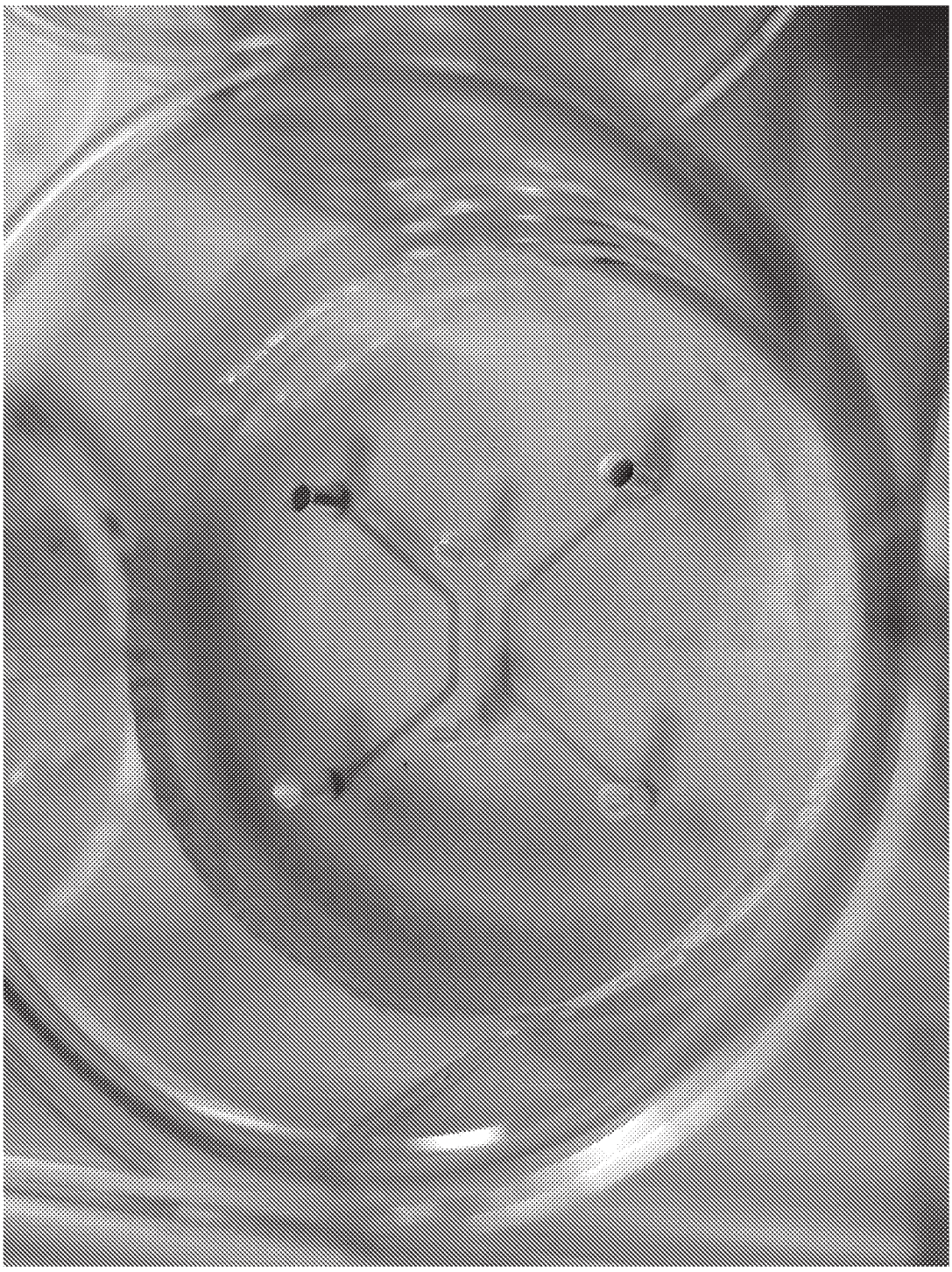


Figure 3

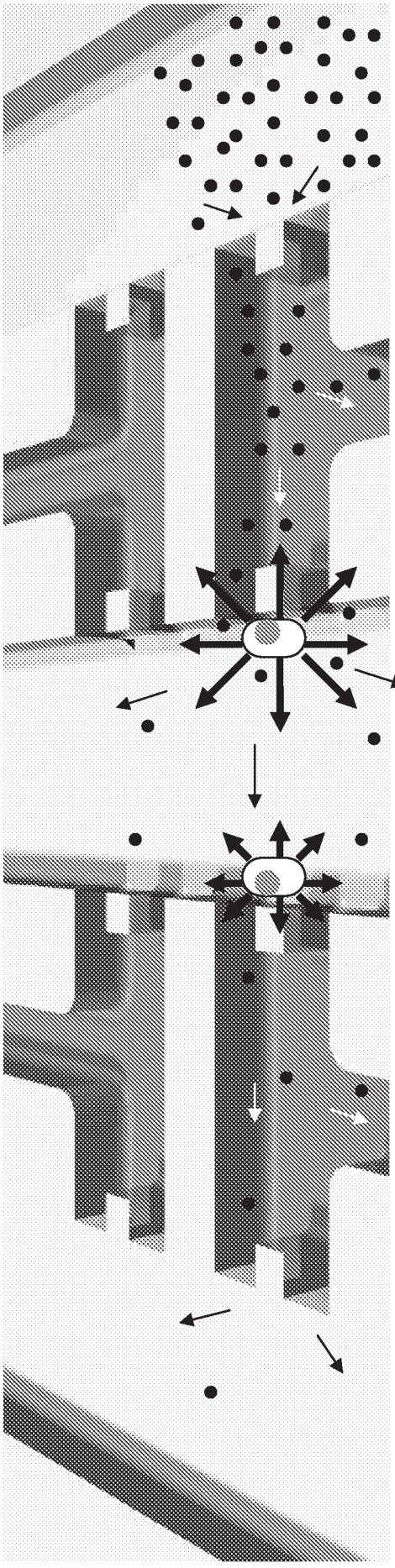


Figure 4A

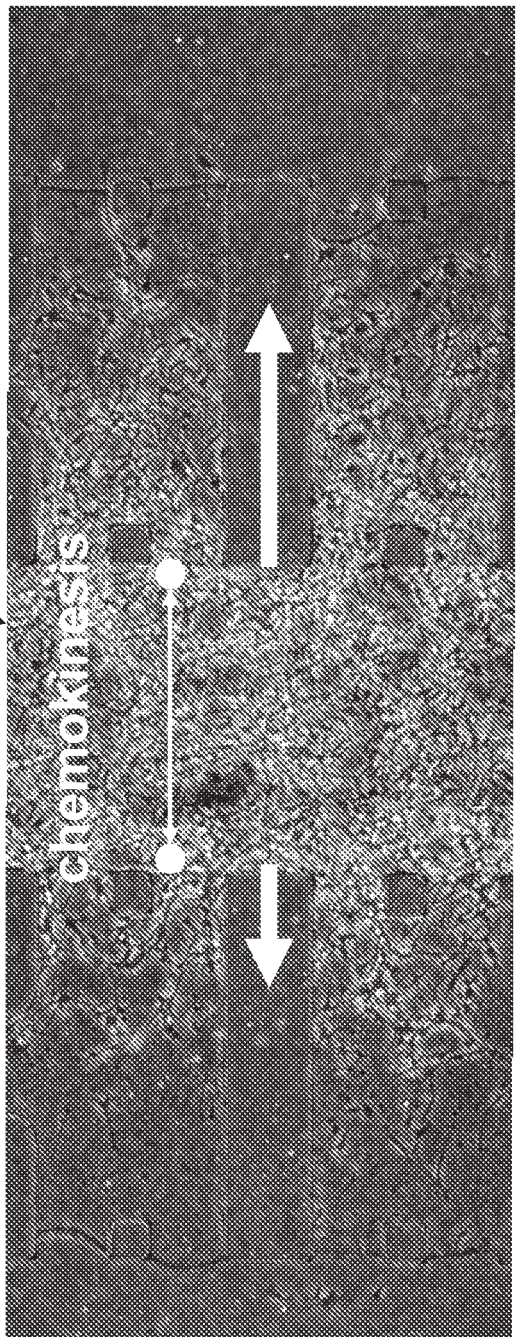


Figure 4B

Figure 4

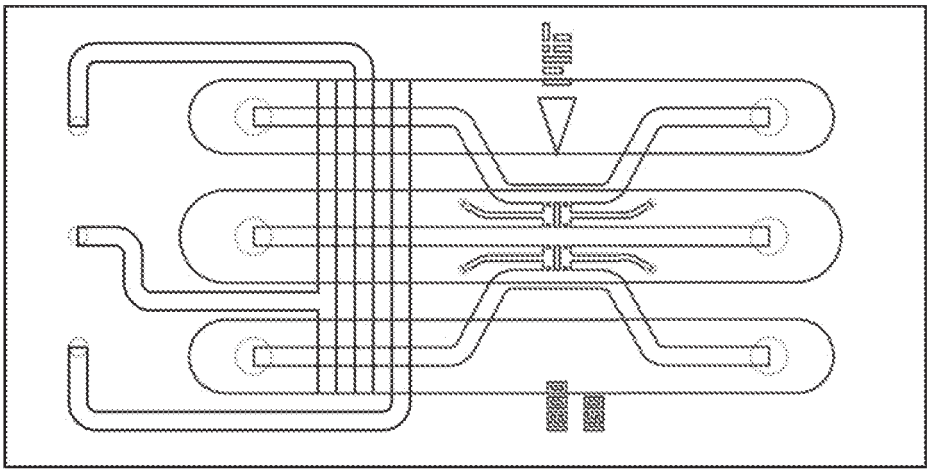


Figure 5

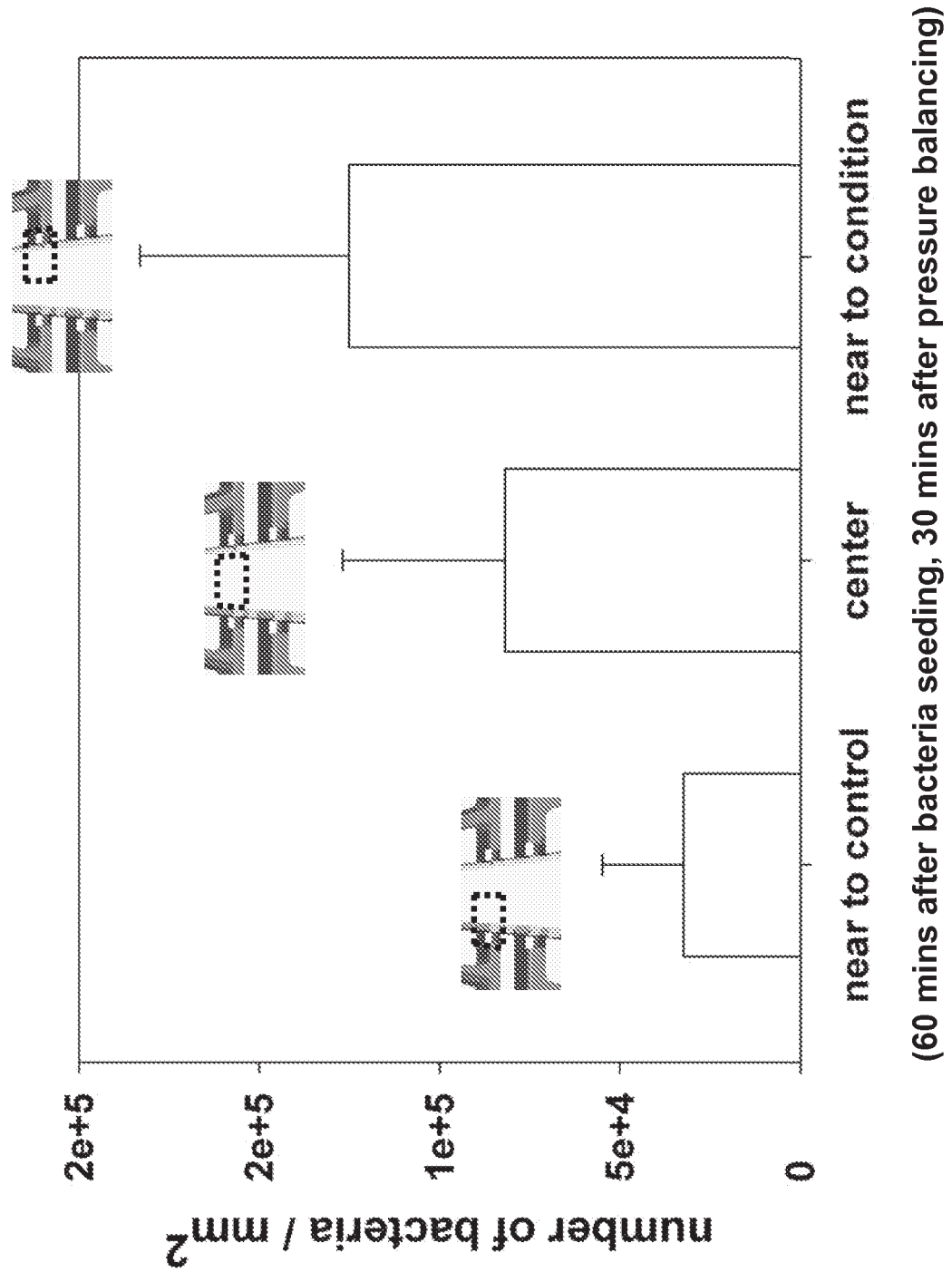

number of bacteria / mm2
2e+5
2e+5
1e+5
5e+4
0
near to control
center
near to condition
(60 mins after bacteria seeding, 30 mins after pressure balancing)

Figure 6

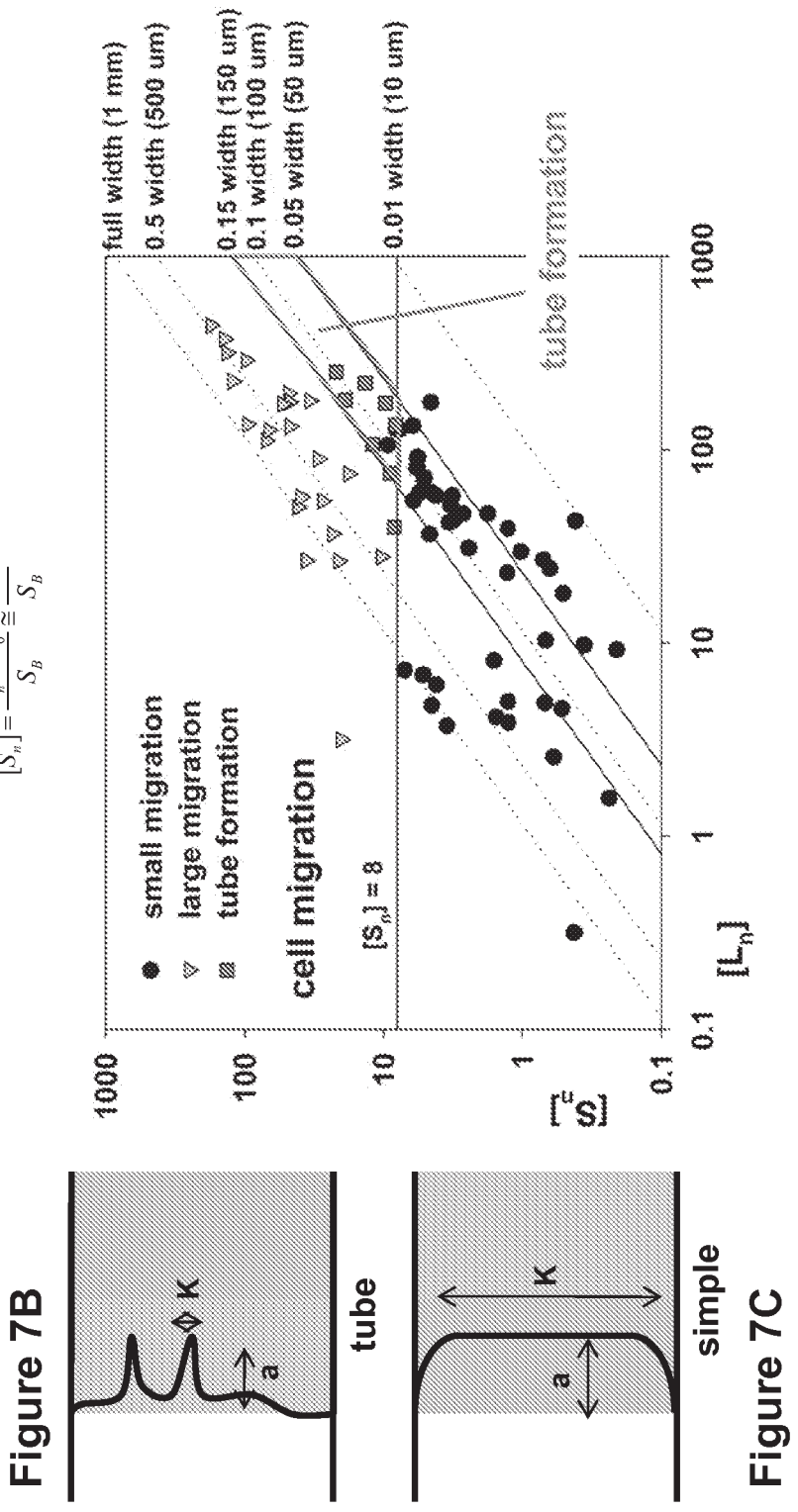


Figure 7A

Figure 7B

Figure 7C

Figure 7

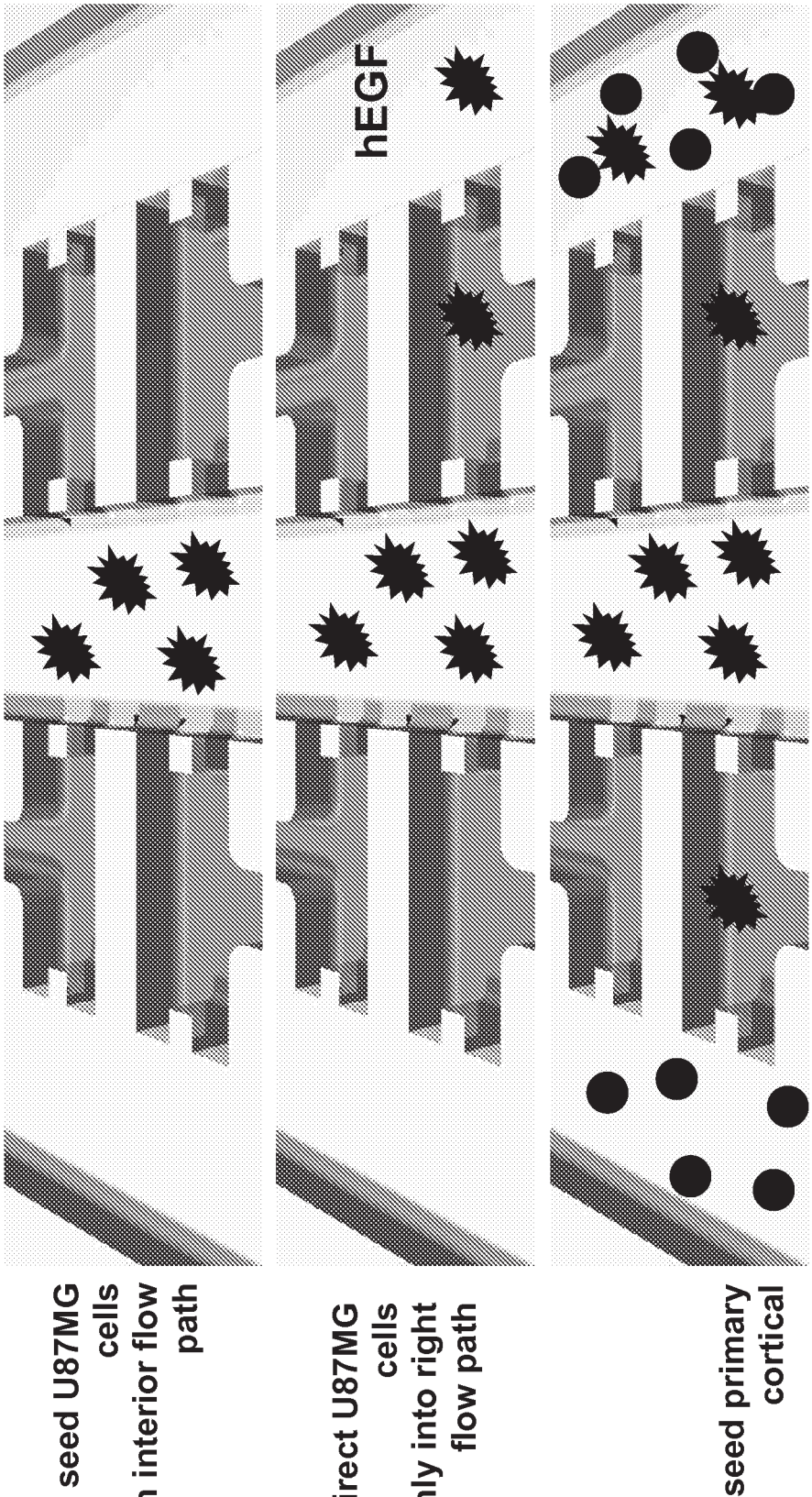
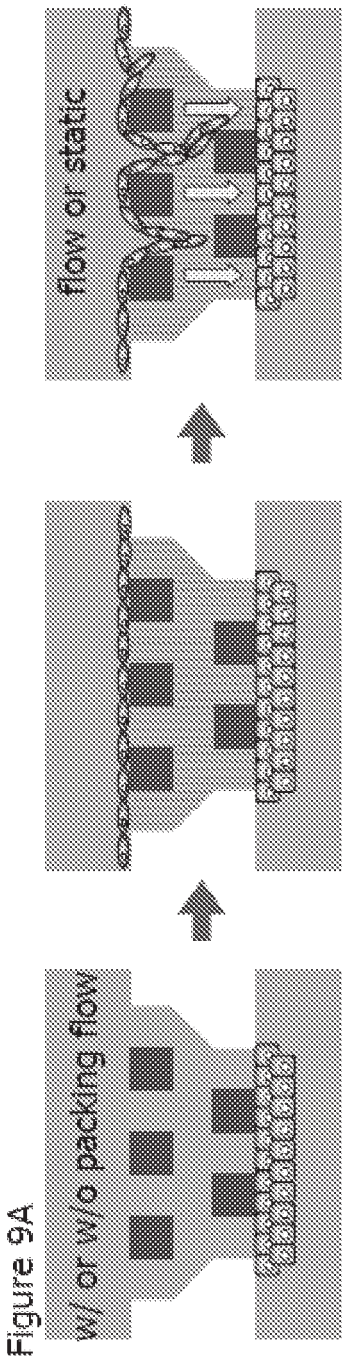

seed U87MG cells in interior flow path
direct U87MG cells only into right flow path
hEGF
seed primary cortical

Figure 8


Figure 9A
w/ or w/o packing flow
flow or static

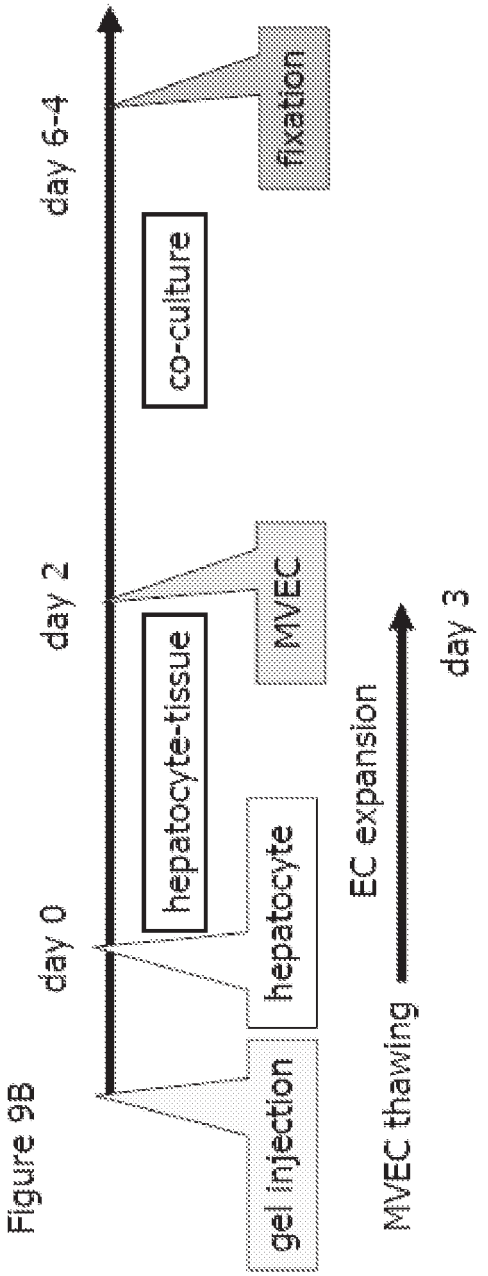
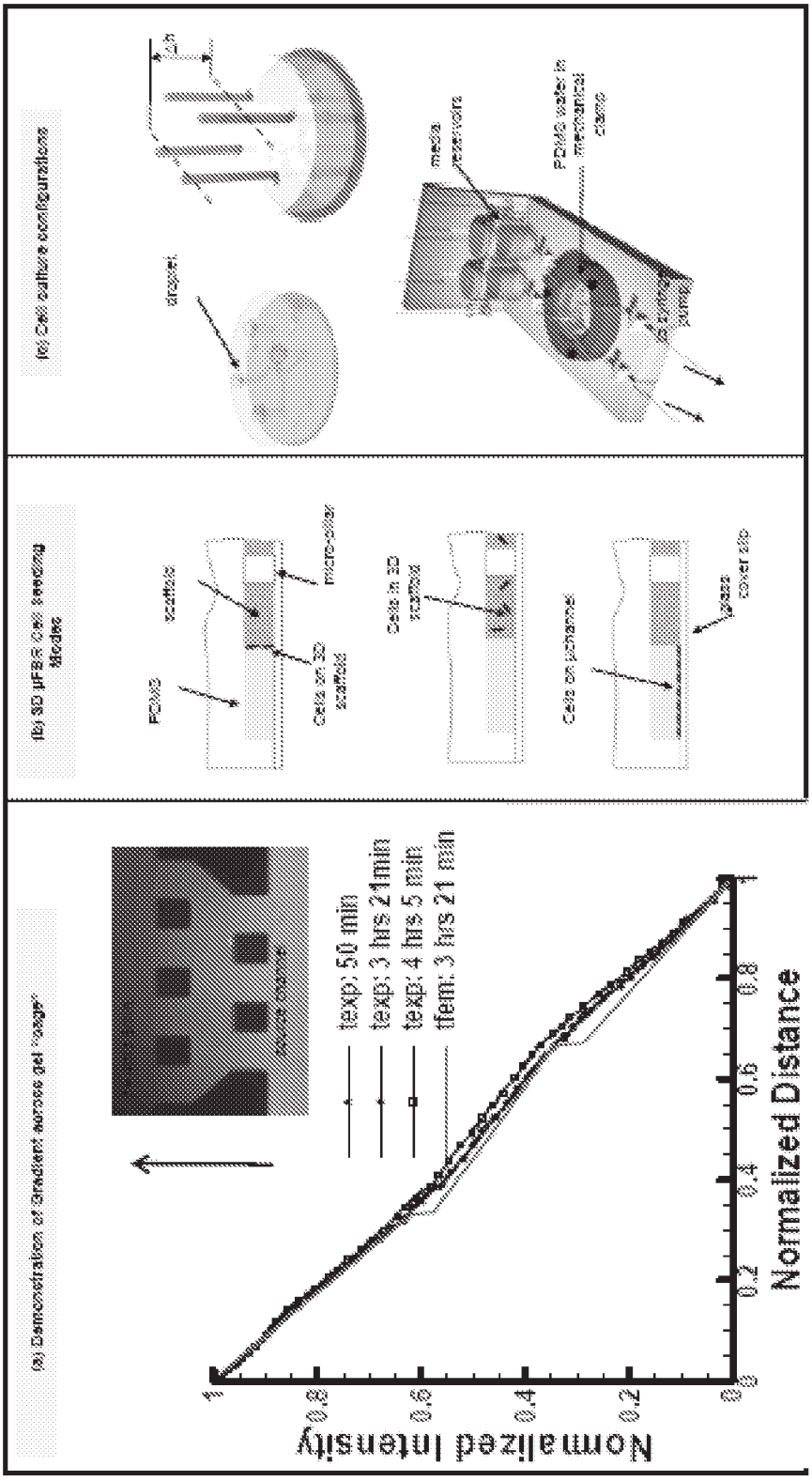

Figure 9B
day 0
day 2
day 6-4
hepatocyte-tissue
co-culture
fixation
MVEC
hepatocyte
gel injection
EC expansion
MVEC thawing
day 3

Figure 9



Figure 10

Figure 11


Figure 11A
Figure 11B
Figure 11C
Normalized Intensity
Normalized Distance
texp: 50 min
texp: 3 hrs 21min
texp: 4 hrs 5 min
tfem: 3 hrs 21 min
PDMS
scaffolds
Cells on 3D scaffold
Cells in 3D scaffold
Cells on substrate
glass
cover slip
droplet
media
PDMS wafer in microfluidic device

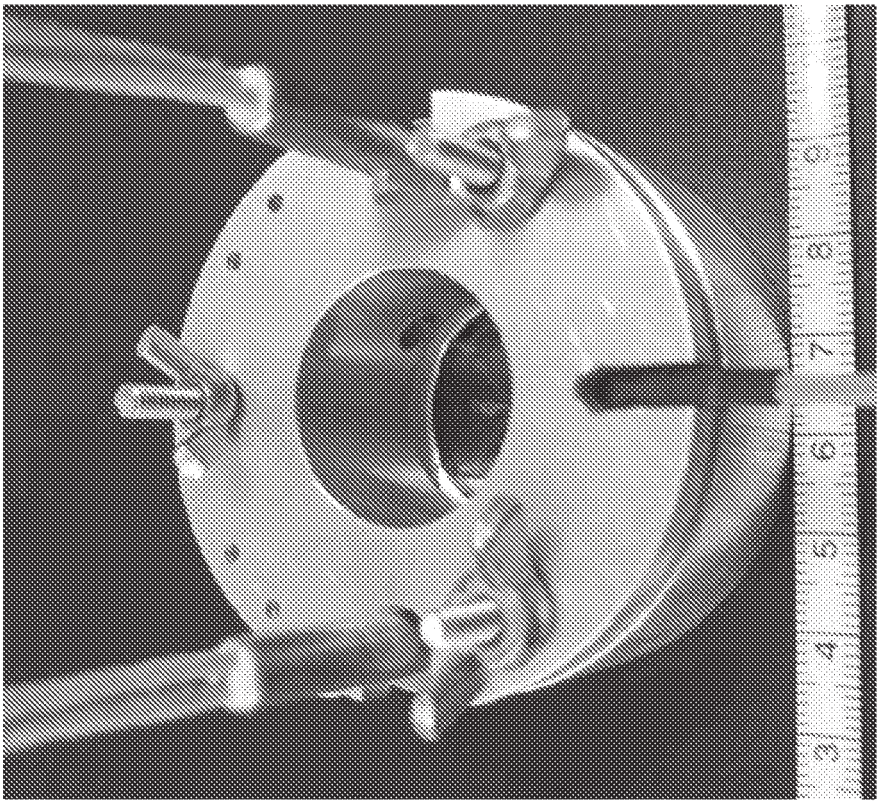


Figure 12B

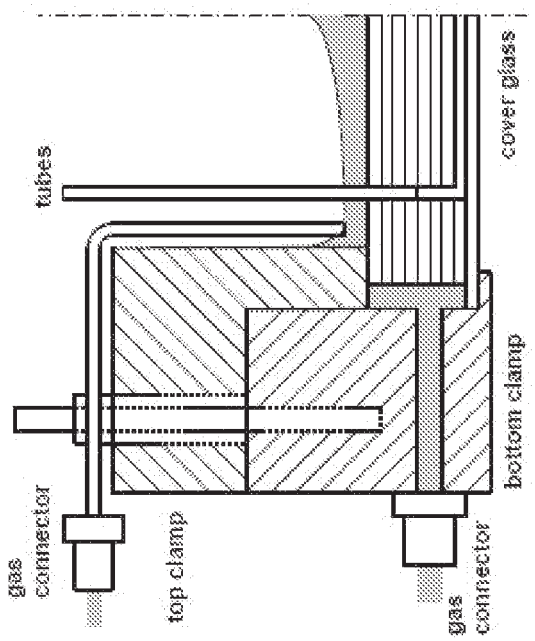


Figure 12A

Figure 12

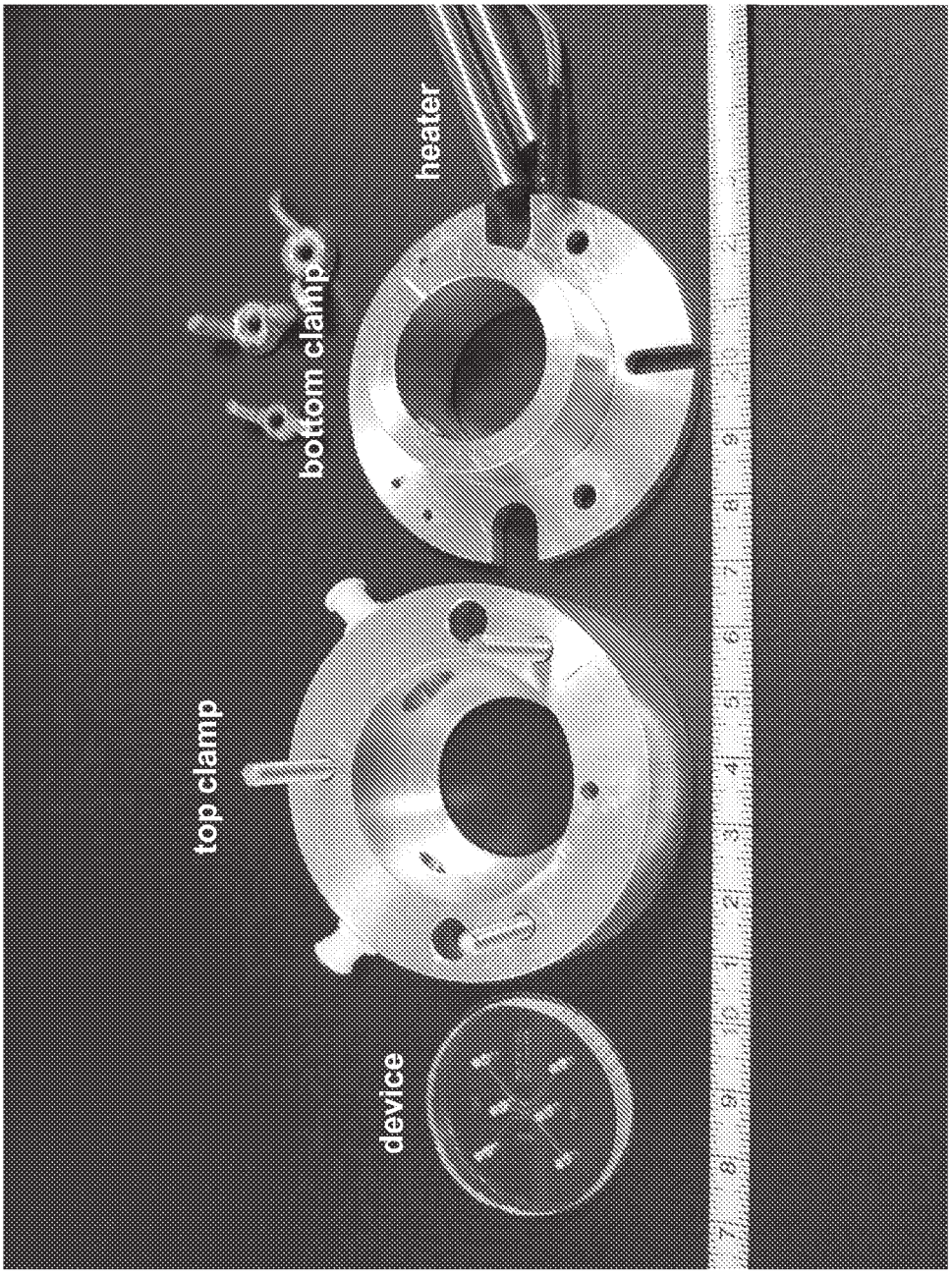


Figure 13

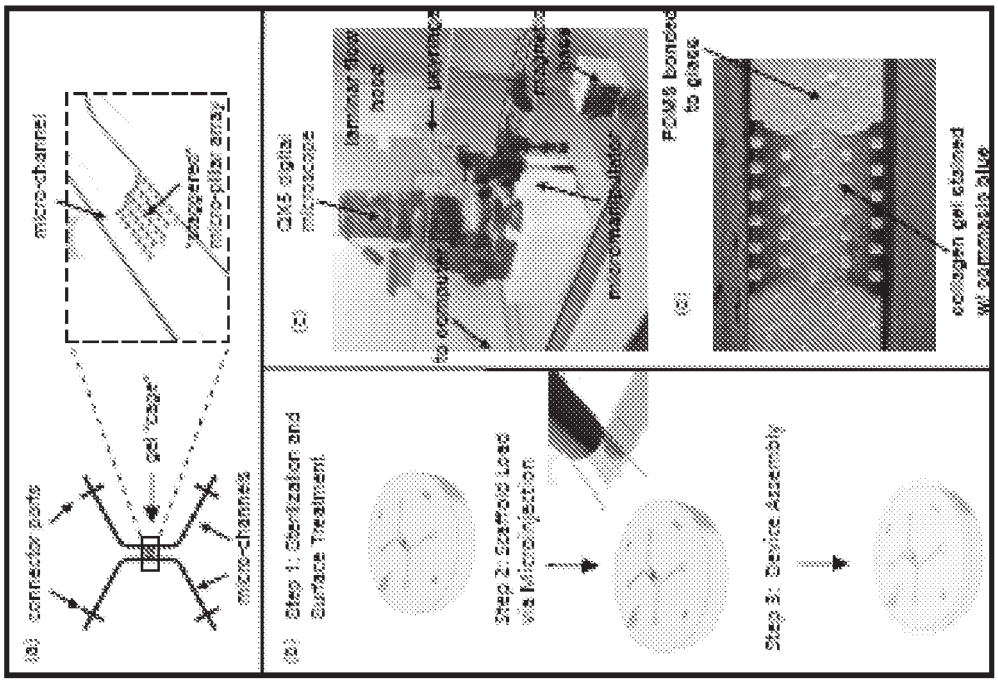

(a) connector ports
micro-channel
gel region
micro-channels
micro-pillar array
(b) Step 1: Sterilization and Surface Treatment
Step 2: Scaffold Loading via Micropipetting
Step 3: Device Assembly
(c) CMS digital microscope
(d)
PDMS bonded to glass
collagen gel stained w/ fluorescein dye

Figure 14

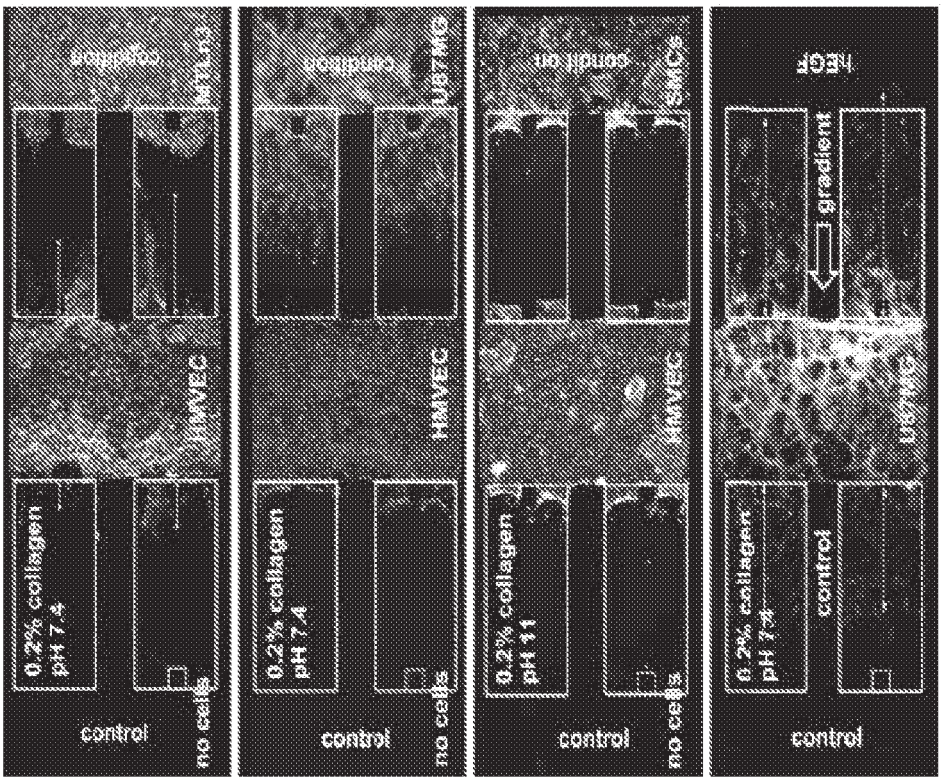
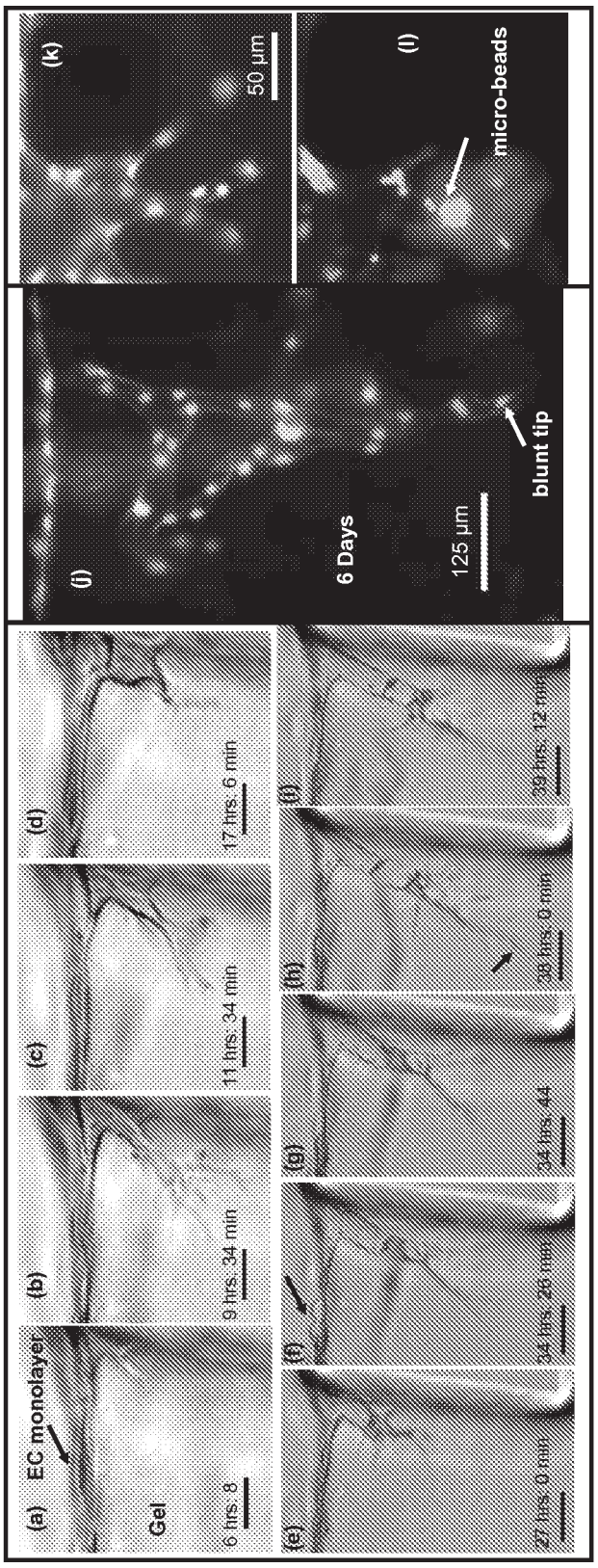

0.2% collagen
pH 7.4
control
no cells
HMVEC
fibroblast
0.2% collagen
pH 11
control
no cells
HMVEC
smooth mu
SMCs
0.2% collagen
pH 7.4
control
HMVEC
fibroblast
gradient

Figure 15

Figure 16


(a)
EC monolayer
(b)
(c)
(d)
Gel
6 hrs. 8
9 hrs. 34 min
11 hrs. 34 min
17 hrs. 6 min
(e)
(f)
(g)
(h)
(i)
27 hrs. 0 min
34 hrs. 26 min
34 hrs. 44
38 hrs. 0 min
39 hrs. 12 min
(j)
6 Days
125 µm
blunt tip
(k)
50 µm
(l)
micro-beads

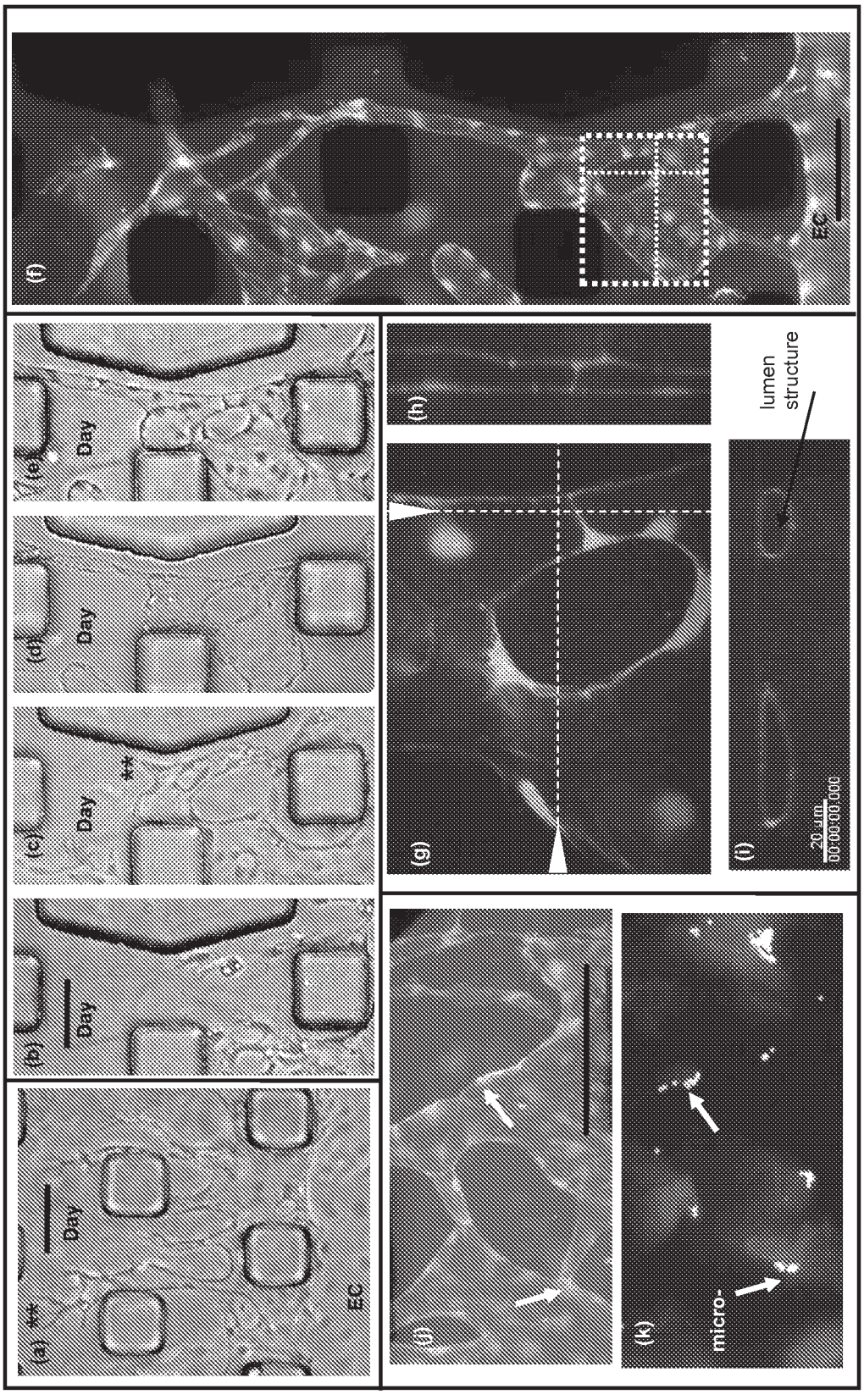

(a)
++
Day
EC
(b)
Day
(c)
Day
++
(d)
Day
(e)
Day
(f)
EC
(g)
(h)
(i)
lumen
structure
20 μm
(j)
(k)
micro-

Figure 17

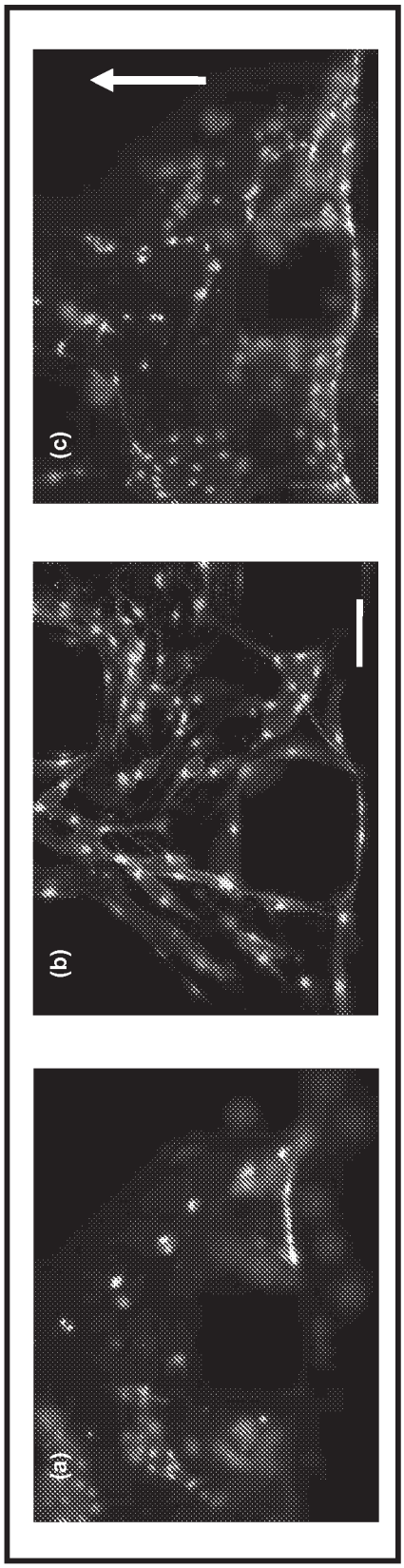


Figure 18

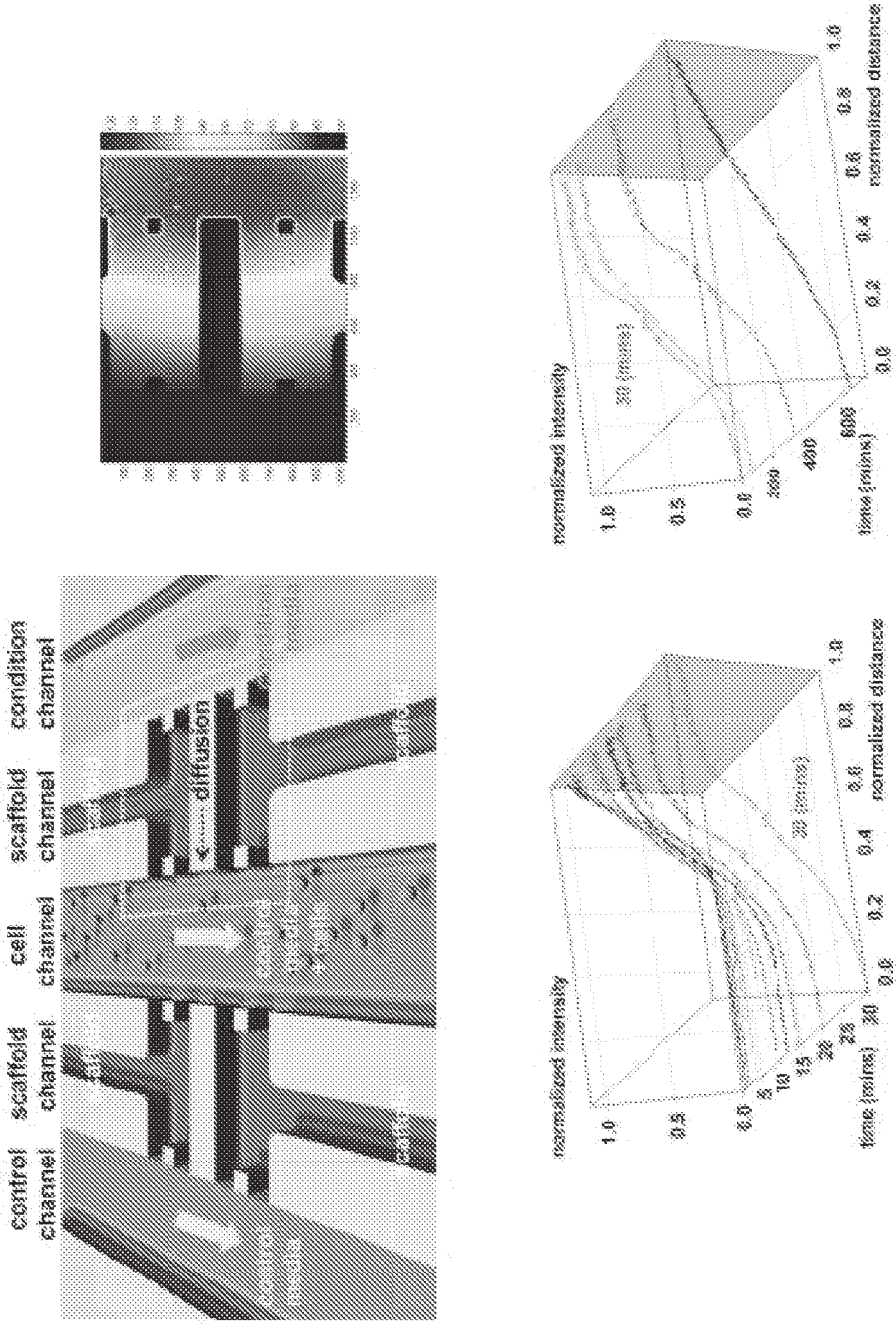

control channel
scaffold channel
cell channel
scaffold channel
condition channel
diffusion
normalized intensity
time (mins)
normalized distance
30 (mins)

Figure 19

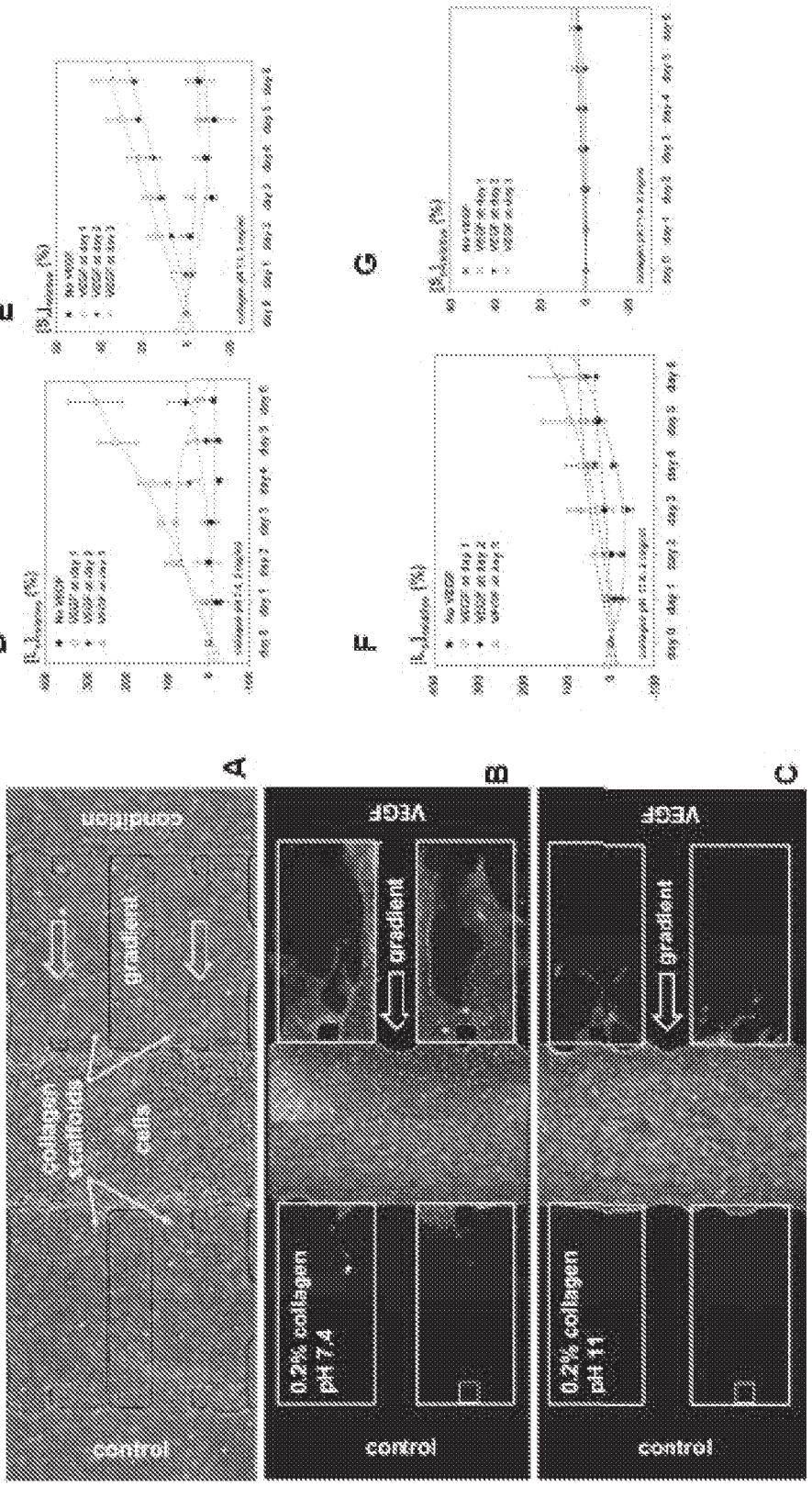

A
B
C
D
E
F
G
control
collagen scaffolds
cells
gradient
condition
VEGF
gradient
control
0.2% collagen pH 7.4
VEGF
gradient
control
0.2% collagen pH 11

Figure 20

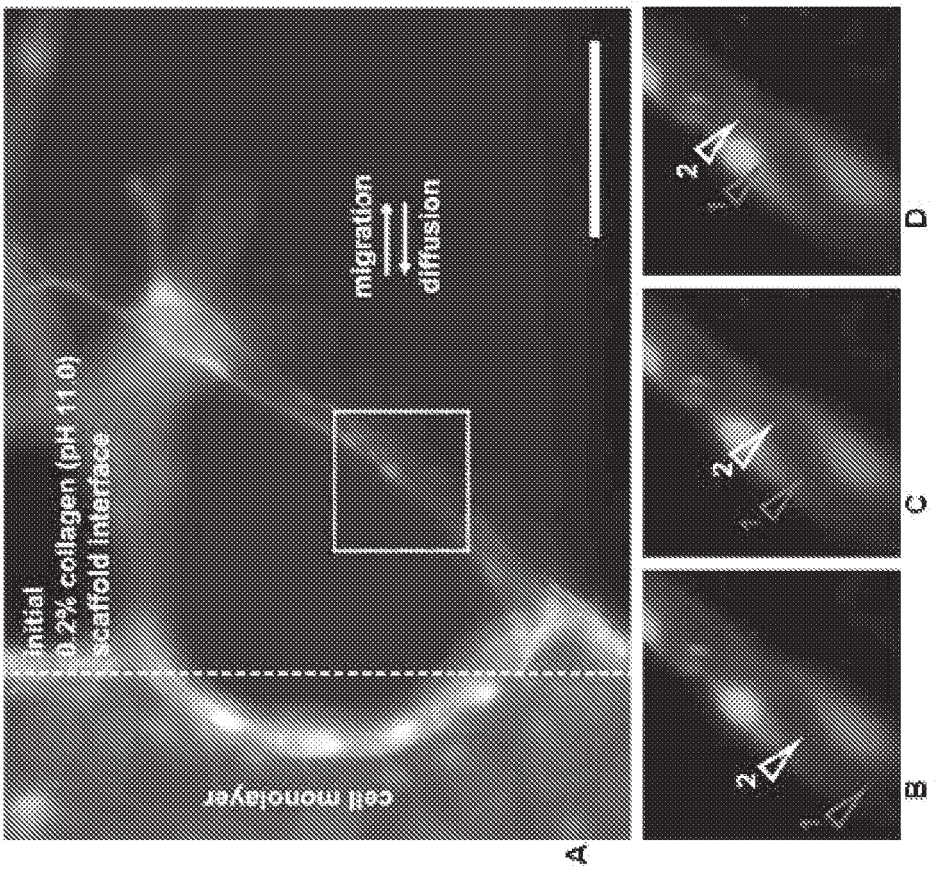

Initial
0.2% collagen (pH 11.0)
scaffold interface
cell monolayer
migration
diffusion
A
B
C
D

Figure 21

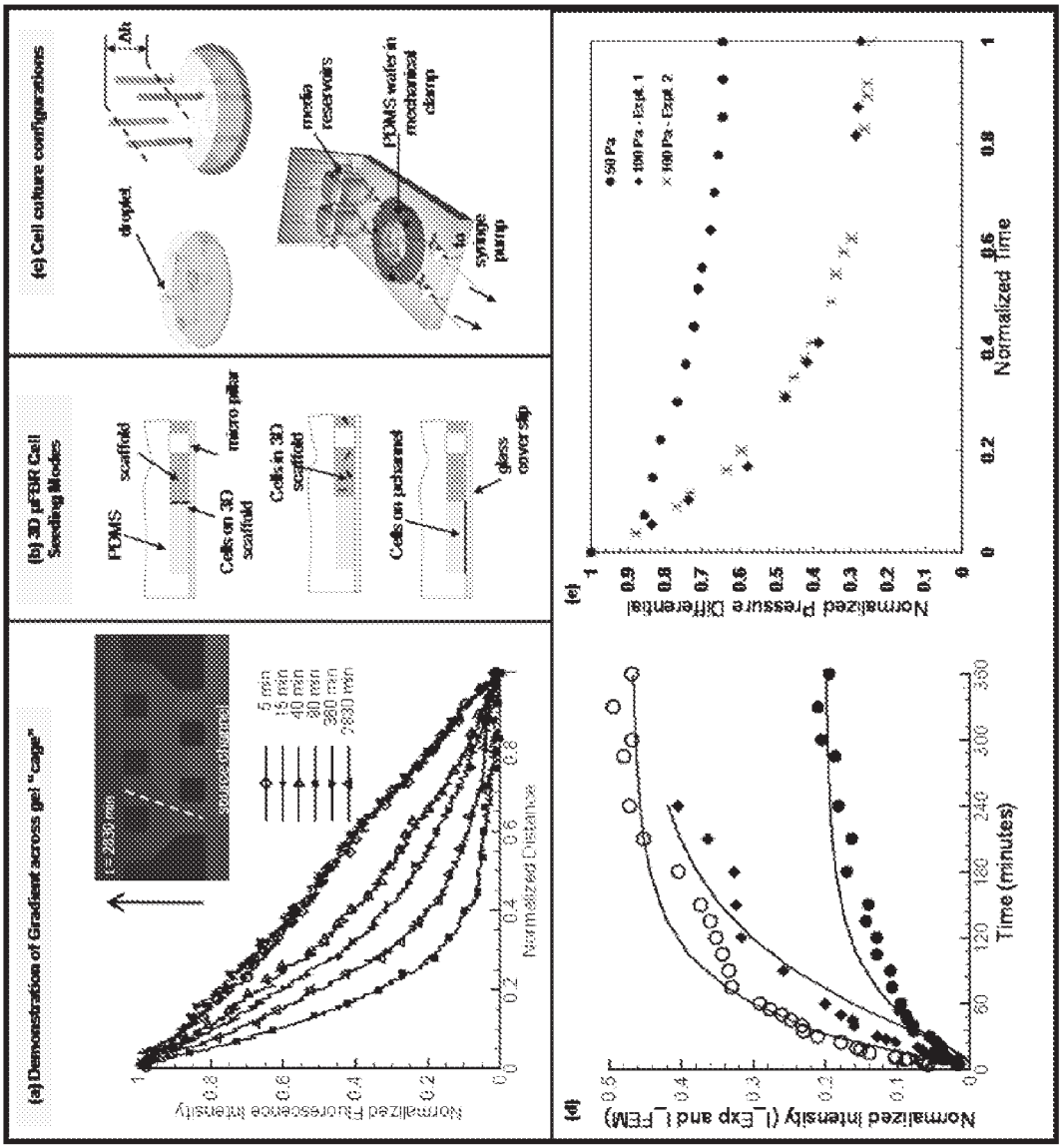

(a) Demonstration of Gradient across gel "cage"
t = 2630 min
5 min
15 min
40 min
80 min
380 min
2630 min
Normalized Fluorescence Intensity
Normalized Distance
(b) 3D pFBR Cell Seeding Modes
PDMS
scaffold
micro pillar
Cells in 3D scaffold
Cells in 3D scaffold
Cells on patterned
glass cover slip
(c) Cell culture configurations
droplet
Δh
media reservoirs
PDMS wafer in mechanical clamp
syringe pump
(d)
Normalized Intensity (I_Exp and I_FEM)
Time (minutes)
(e)
Normalized Pressure Differential
50 Pa
100 Pa - Expt. 1
100 Pa - Expt. 2
Normalized Time

Figure 22

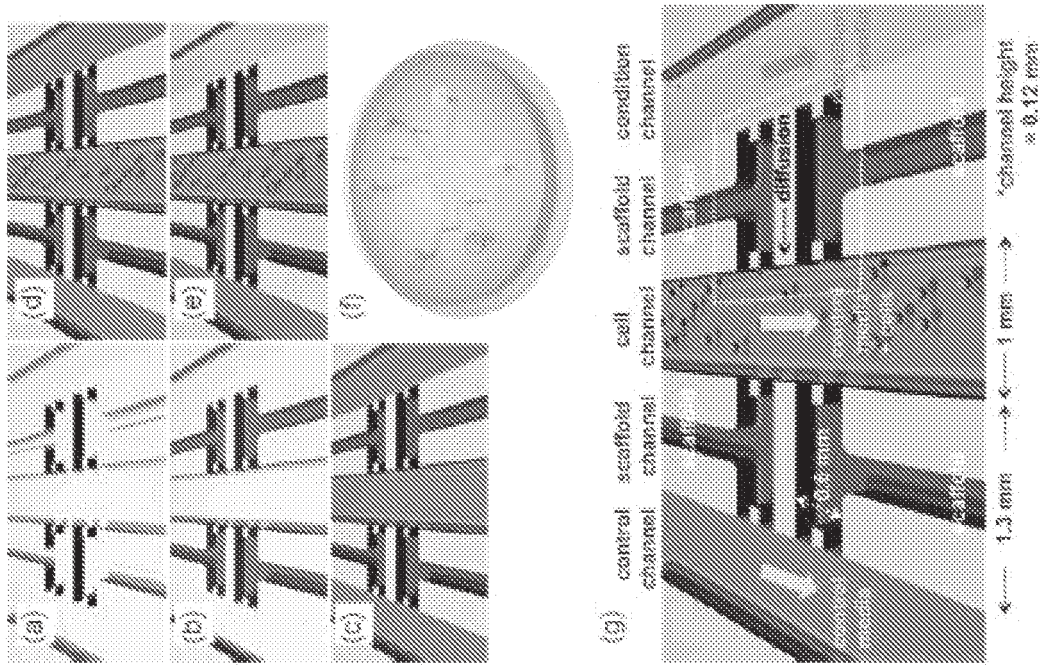

(a)
(b)
(c)
(d)
(e)
(f)
(g)
central channel
scaffold channel
cell channel
scaffold channel
condition channel
diffusion
1.3 mm
1 mm
channel height = 0.12 mm

Figure 23

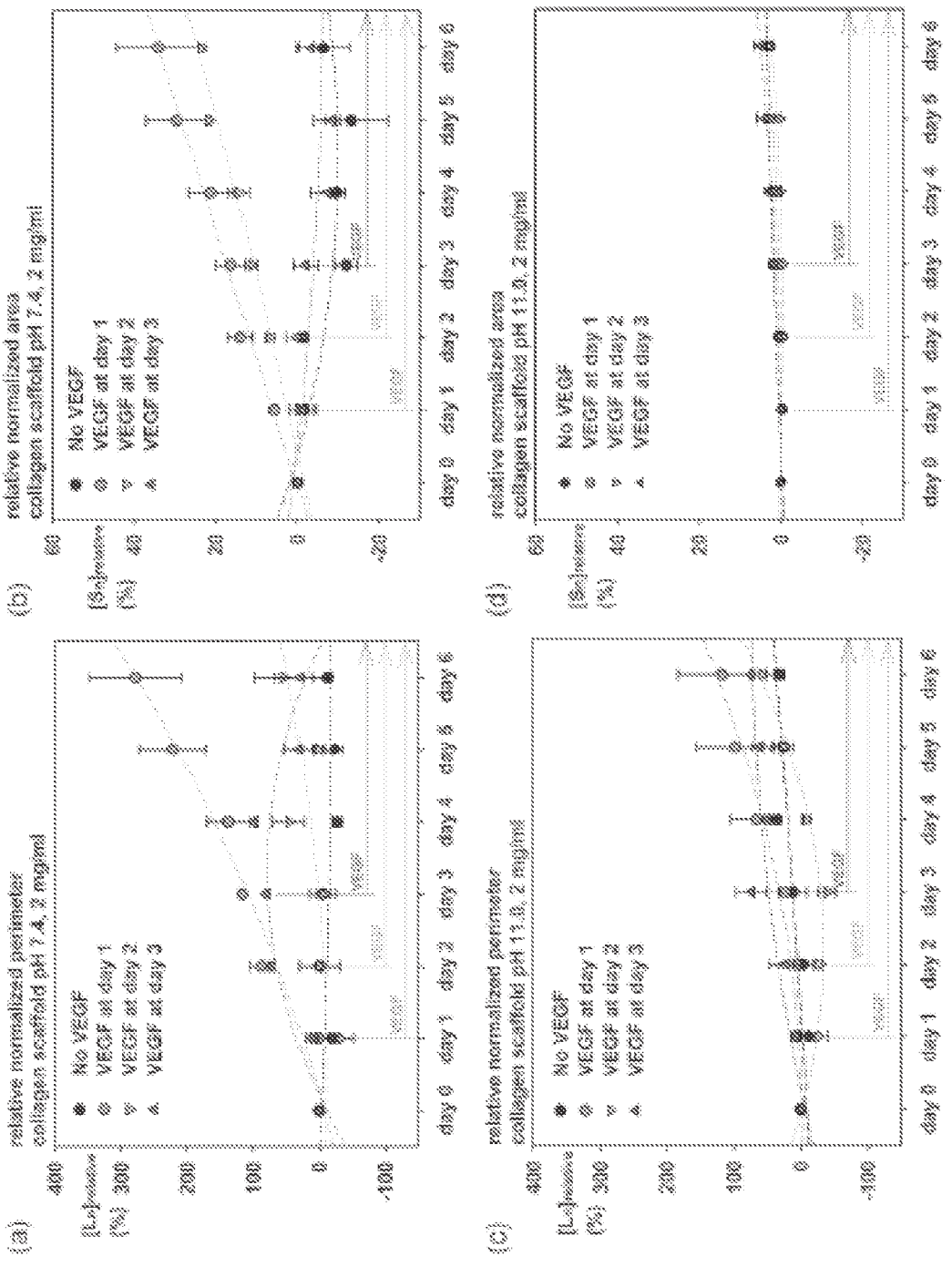

(a)
relative normalized perimeter
collagen scaffold pH 7.4, 2 mg/ml
(b)
relative normalized area
collagen scaffold pH 7.4, 2 mg/ml
(c)
relative normalized perimeter
collagen scaffold pH 11.8, 2 mg/ml
(d)
relative normalized area
collagen scaffold pH 11.8, 2 mg/ml
No VEGF
VEGF at day 1
VEGF at day 2
VEGF at day 3
day 0
day 1
day 2
day 3
day 4
day 5
day 6

Figure 24

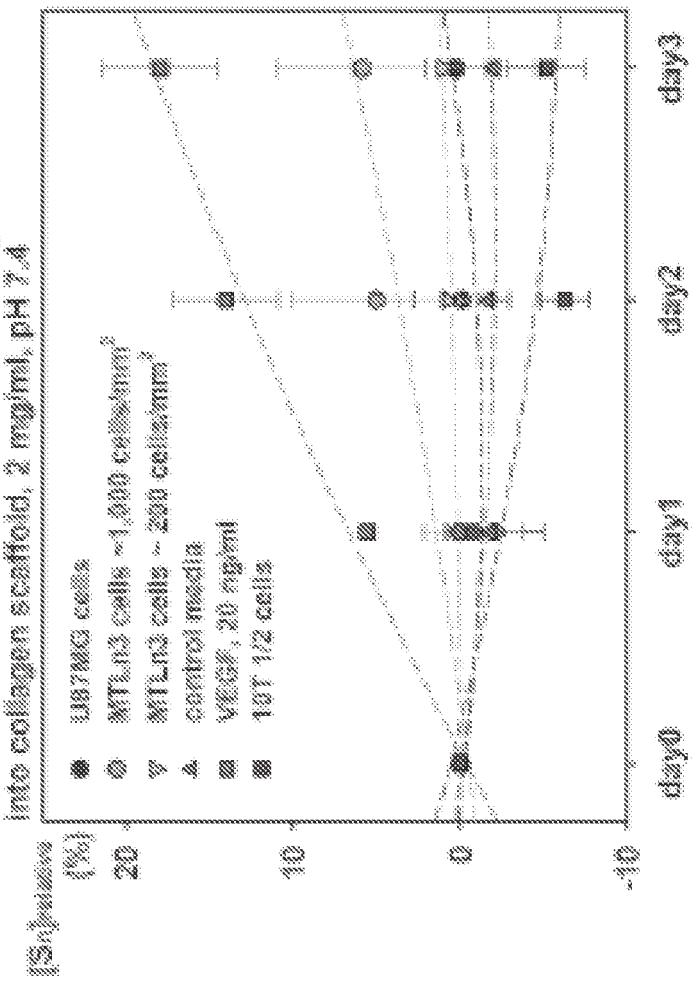

relative normalized area of HMVEC migration into collagen scaffold, 2 mg/ml, pH 7.4
(%)
20
10
0
-10
day0
day1
day2
day3
U87MG cells
MTLn3 cells ~1,000 cells/mm²
MTLn3 cells ~ 200 cells/mm²
control media
VEGF, 20 ng/ml
10T 1/2 cells

Figure 25

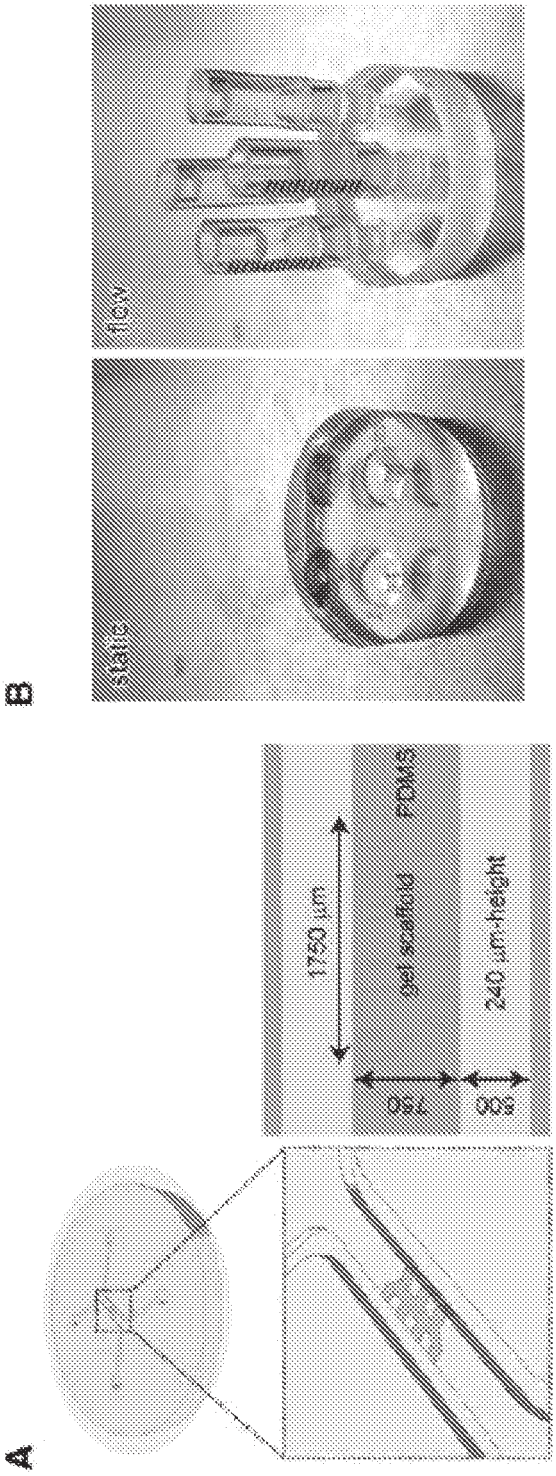


Figure 26

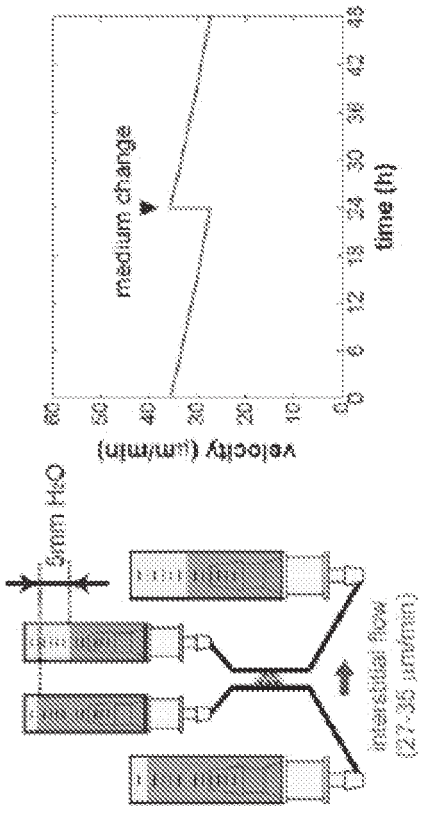

velocity (um/min)
60
50
40
30
20
10
0
0
6
12
18
24
30
36
42
48
time (h)
medium change
3mm H2O
interstitial flow
(27.35 um/min)

Figure 27

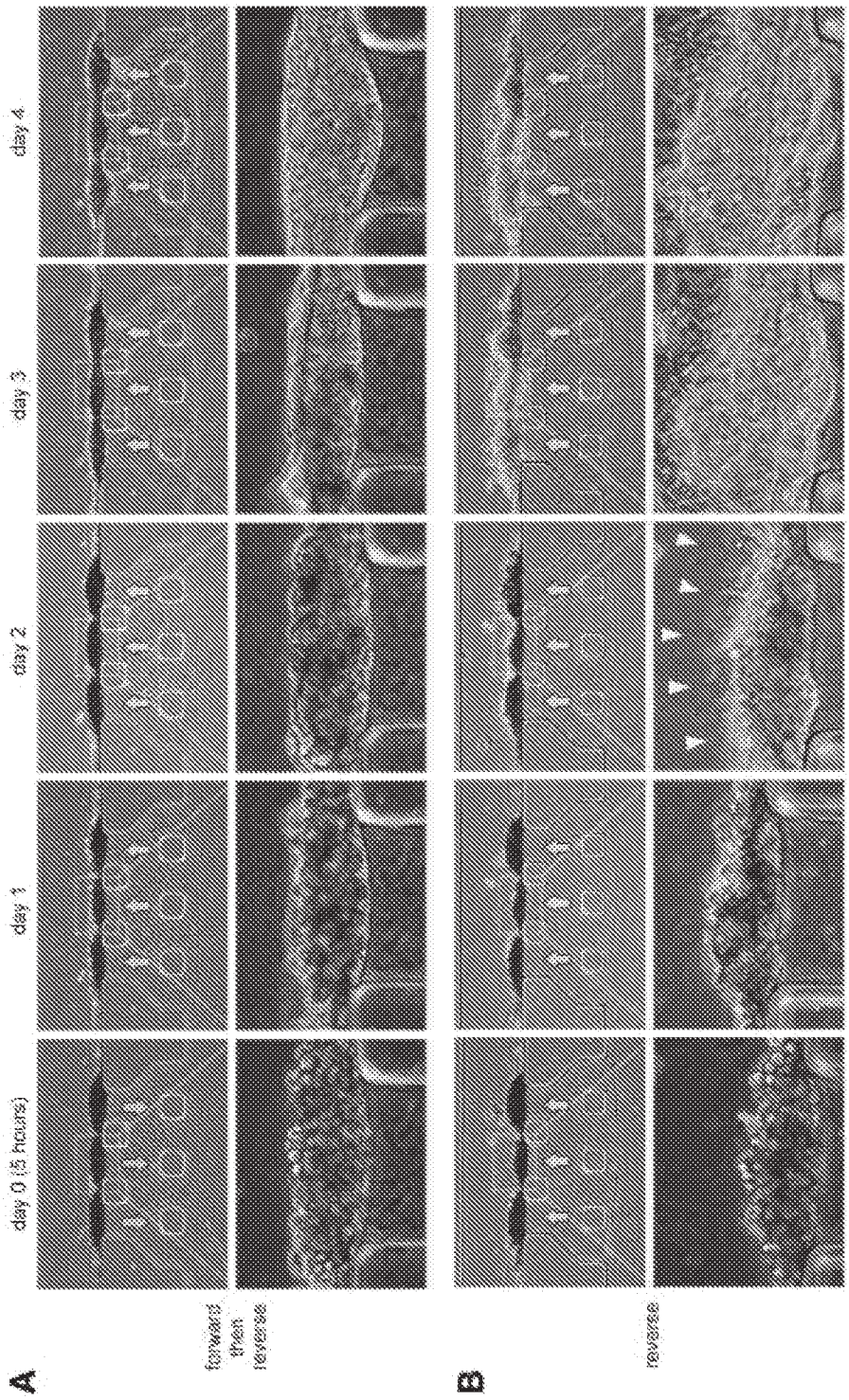

A
day 0 (5 hours)
day 1
day 2
day 3
day 4
forward then reverse
B
reverse

Figure 28

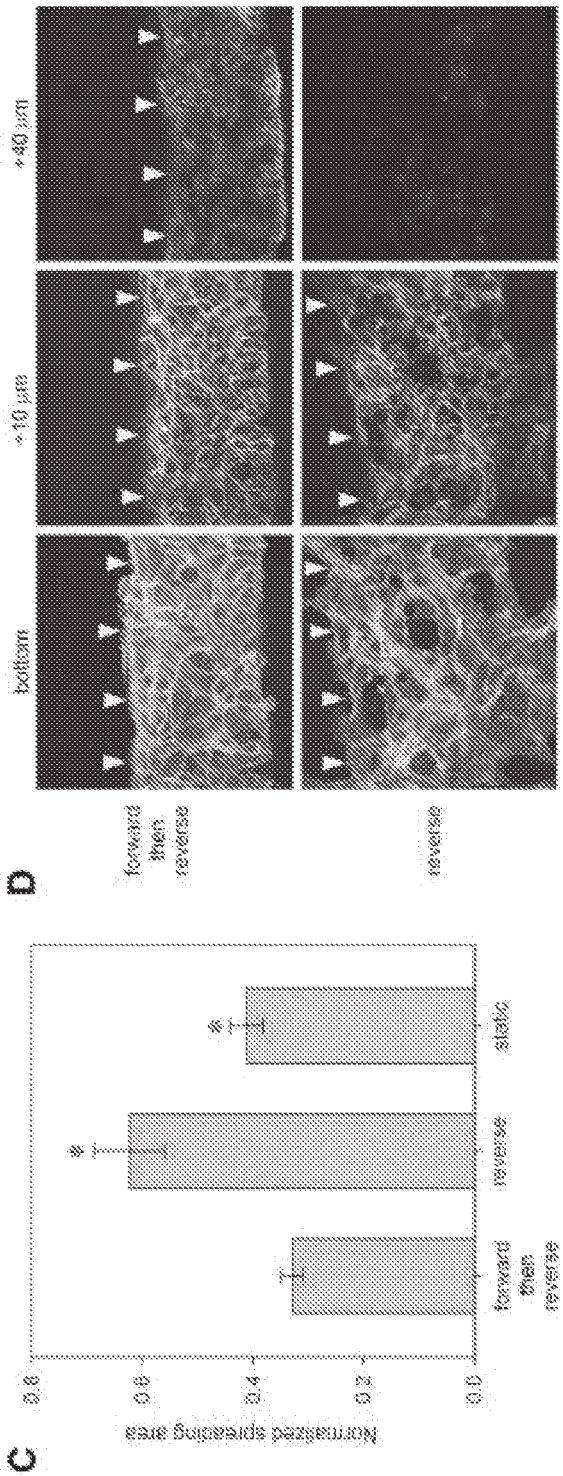

C
Normalized spreading area
0.0
0.2
0.4
0.6
0.8
*
*
forward then reverse
reverse
static
D
bottom
+10 µm
+40 µm
forward then reverse
reverse

Figure 29

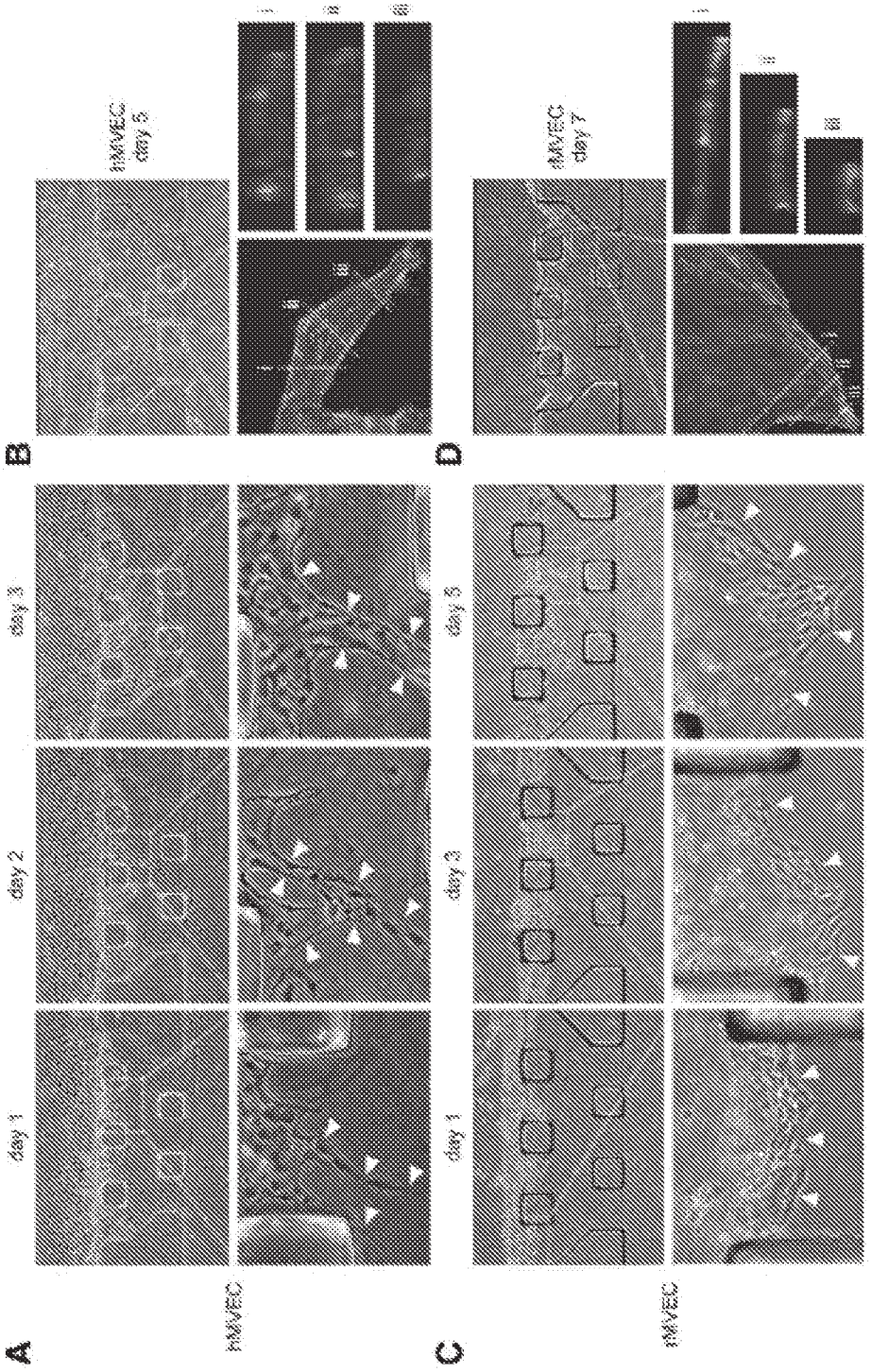

A
day 1
day 2
day 3
hMVEC
B
hMVEC
day 5
C
day 1
day 3
day 5
hMVEC
D
hMVEC
day 7

Figure 30

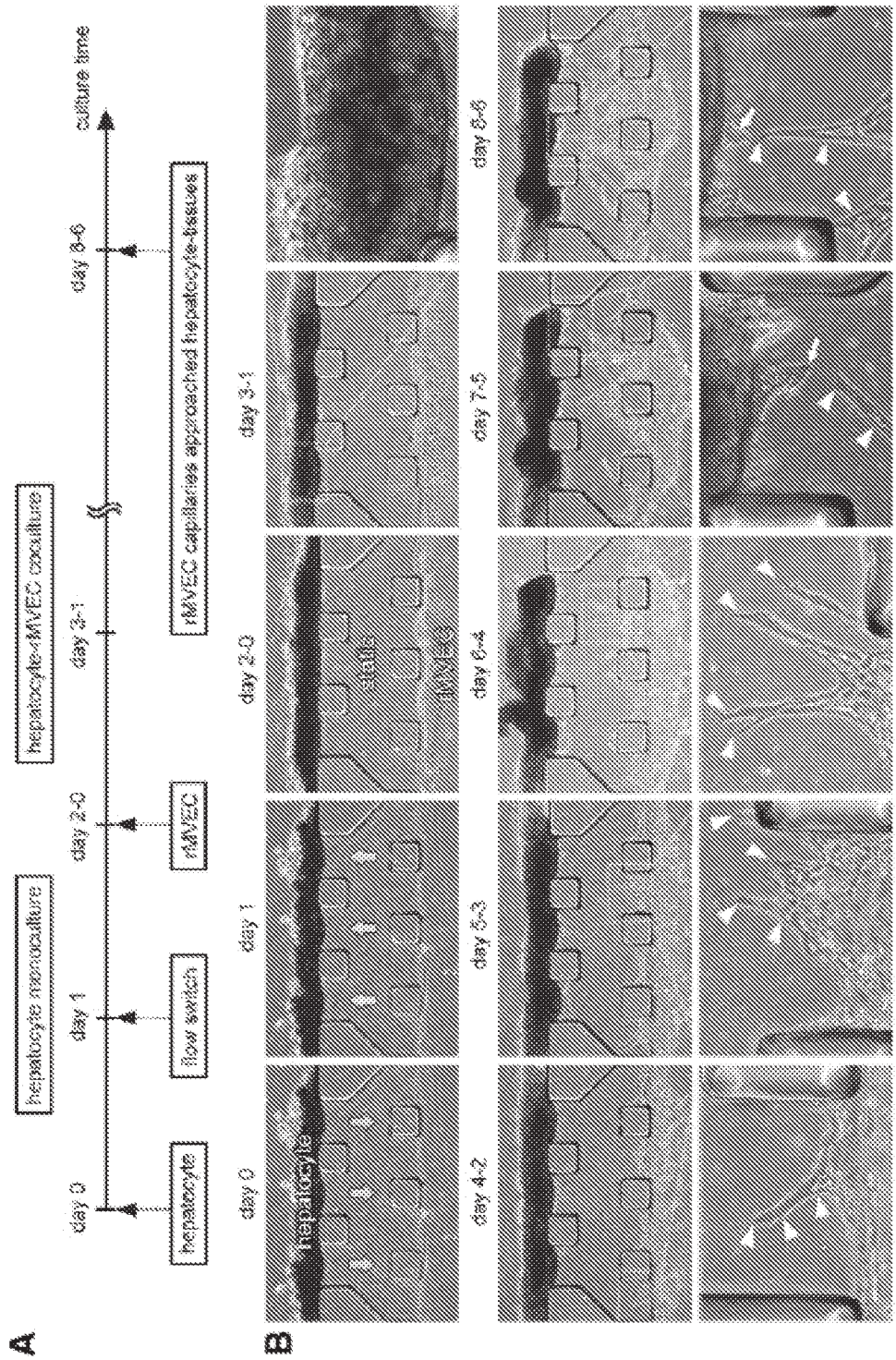
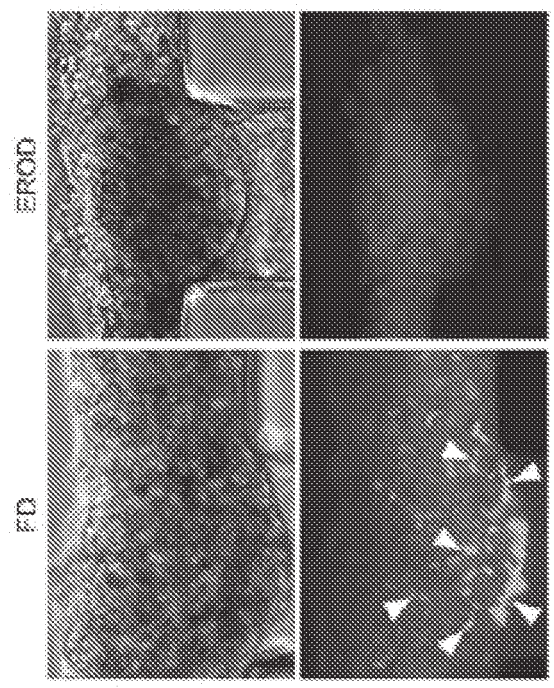

A
hepatocyte monoculture
hepatocyte-rMVEC coculture
day 0
day 1
day 2-0
day 2-1
day 6-6
culture time
hepatocyte
flow switch
rMVEC
rMVEC capillaries approached hepatocyte-tissues
B
hepatocyte
day 0
day 1
day 2-0
day 3-1
day 4-2
day 5-3
day 6-4
day 7-5
day 8-6
acute
MVEC

Figure 31


FD
EROD

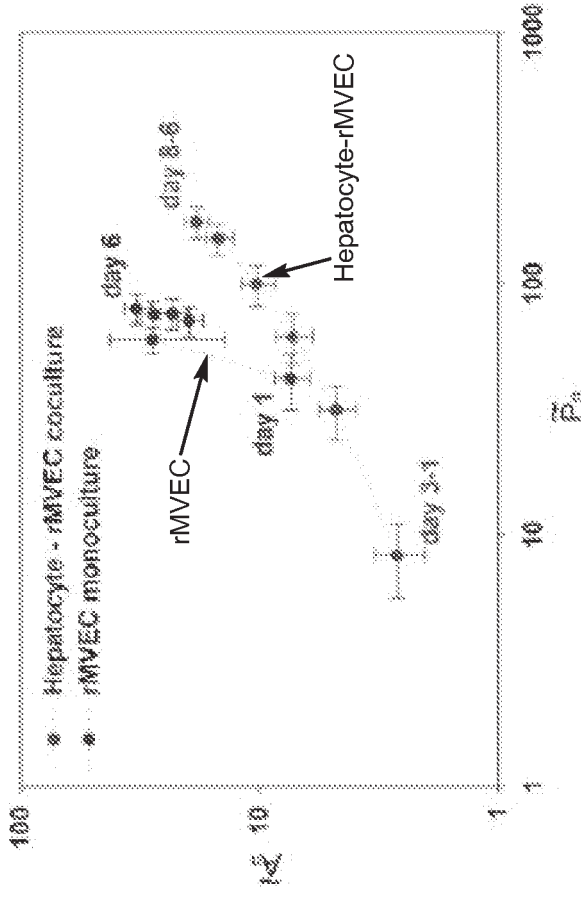

Hepatocyte - rMVEC coculture
rMVEC monoculture
day 6
day 8-6
day 1
day 3-1
rMVEC
Hepatocyte-rMVEC
100
10
1
1
10
100
1000

Figure 32

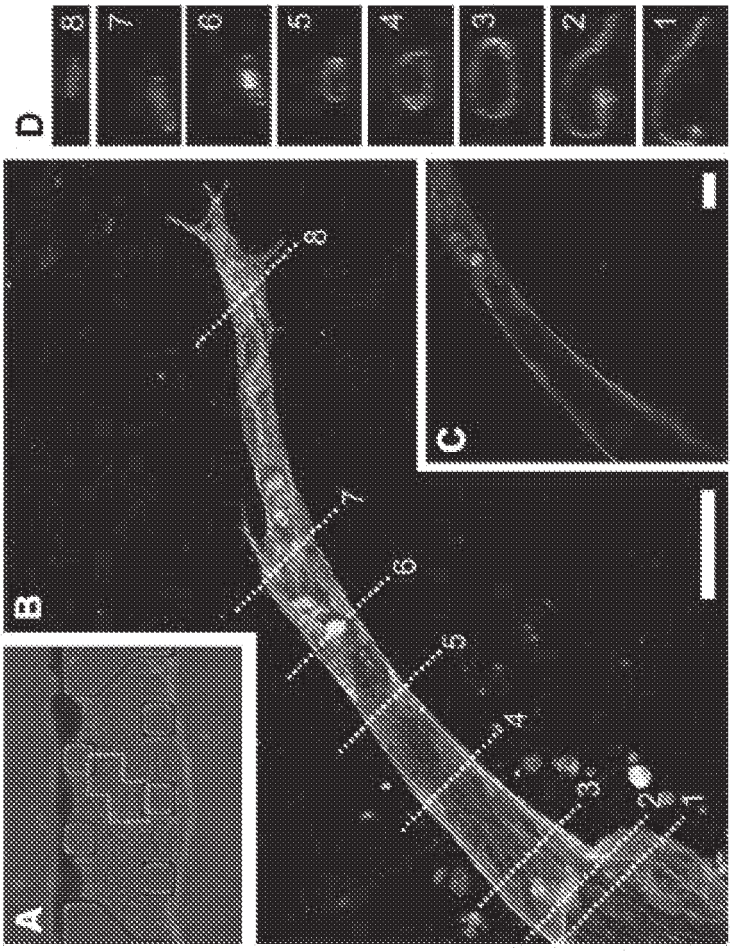


Figure 33

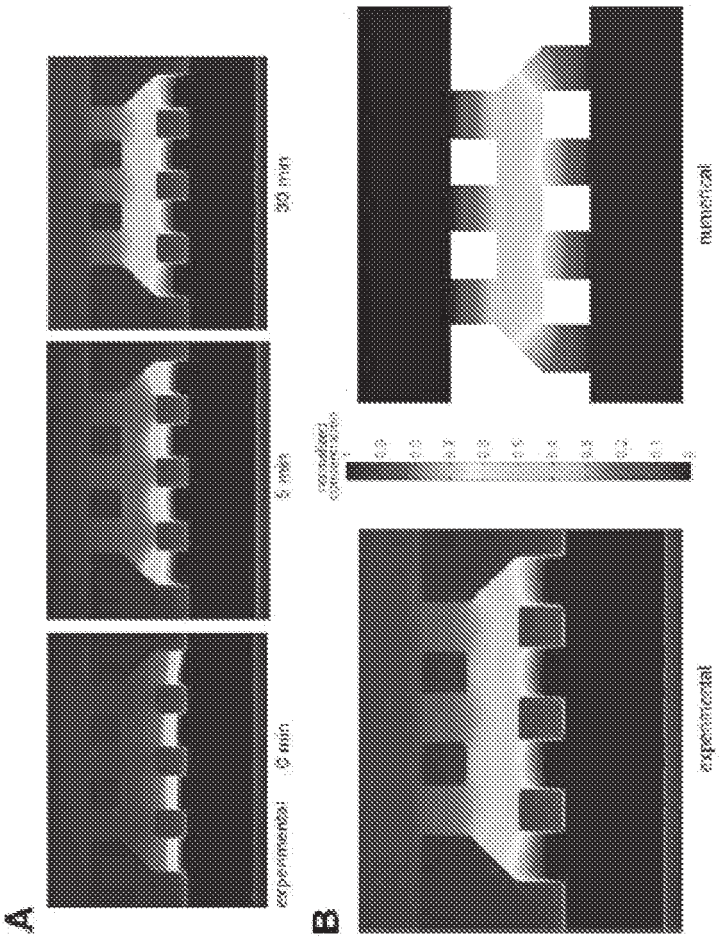


Figure 34