

Supplemental figure 1. Cell density on collagen coated (control) transwells versus flat gelatin and deep gelatin grooves after three days in ALI culture. BEAS2B seeded on collagen coated transwells (A), flat gelatin (B) and deep grooved gelatin (C) for 24 hours of submerged culture + 72 hours of ALI showed no significant difference in nuclear density (D). Error bars represent standard error of the mean. Scale bars are 50µm.

Supplemental figure 2. Atomic force microscopy of PDMS grooves. PDMS grooves replica moulded from diffraction grating were found to have a depth of 162nm and a pitch of 1µm.

Supplemental figure 3. Swelling of gelatin gel of various concentrations after fixing with various concentration of GTA. (A) Experimental design of swelling studies. (B) Swelling ratios of the various gelatin gels versus the various GTA concentrations; the gel used throughout the cell experiments had a swelling ratio of $-4.247 \pm 0.202\%$. Error is standard error of the mean with an $n=3$.

Supplemental figure 4. BEAS2B grown on symmetric PDMS grooves (Pitch of 1µm) with different depths for 72 hours of submerged culture. F-actin staining of cells on deep (3.14µm deep) grooves (A) and shallow (600nm deep) grooves (B). Grooves with a depth of 3.14µm showed significantly less angular deviation than symmetric grooves with a depth of 600nm (C). Angular histograms corresponding to the above substrate condition quantify cell alignment on substrates, where the red line indicates the direction of the grooves. Error bars represent standard error of the mean and *indicates significant difference (where $p = 0.004$). Scale bars are 50µm.

Supplemental figure 5. F-Actin of BEAS2B on gelatin inserts during 24 hours submerged culture plus 48 hours ALI. BEAS2B on flat (A) and shallow microgrooved (B) gelatin inserts are not aligned after 48 hours of ALI. However, they show similar levels of

apicobasal polarization as evidenced by cilia formation (C). Angular histograms corresponding to the above substrate condition quantify cell alignment on substrates, where the red line indicates the direction of the grooves. Error bars represent standard error of the mean. Scale bars are 50um.

Supplemental figure 6: Acetylated tubulin staining of cilia in BEAS2B cells after 24h of ALI culture on standard transwell filters. Scale bars are 50um.

Supplemental figure 7. Deep grooved gelatin insert characterization after ALI culture.

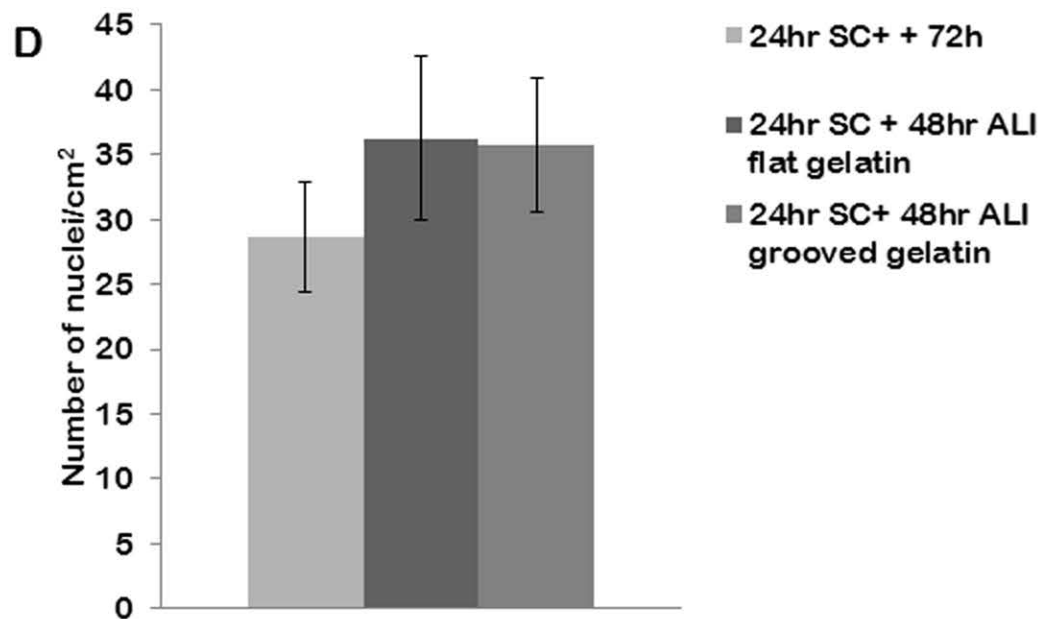
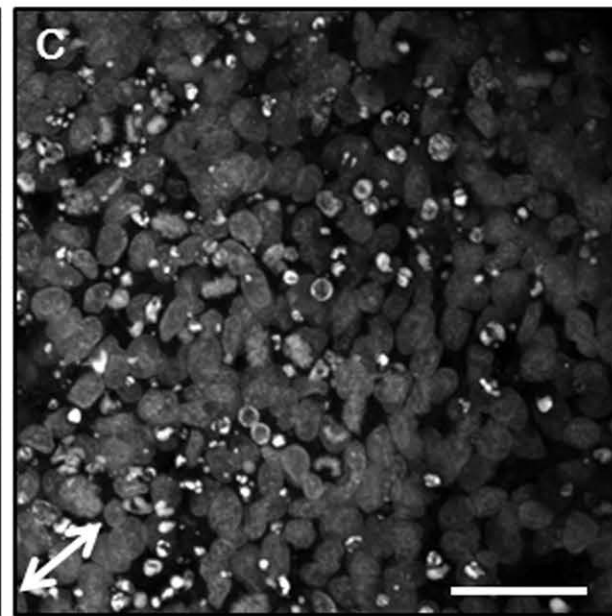
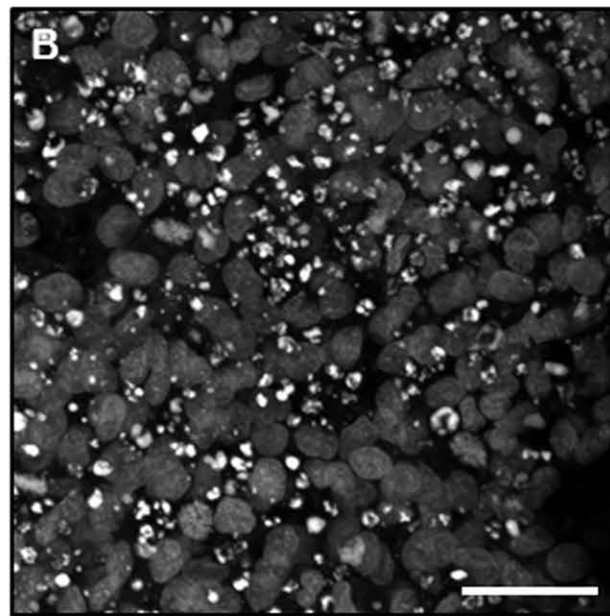
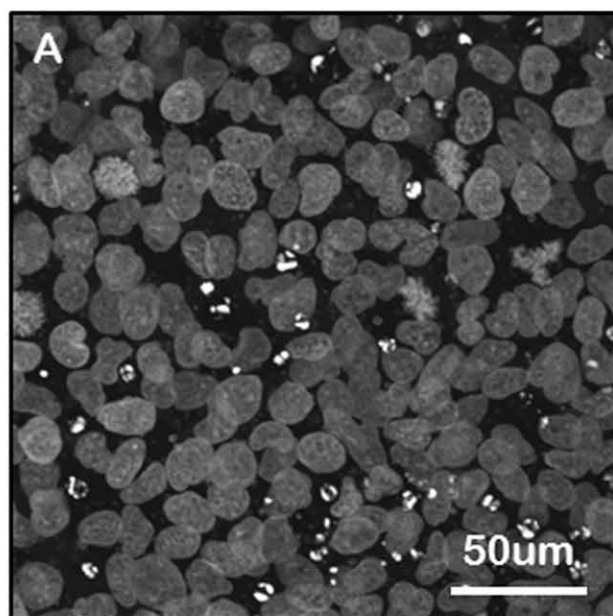
After 72 hours of submerged culture, BEAS2B stained with FITC-Phalloidin (A and C) showed the grooves en face. In (C) in particular, the difference between grooved and flat areas was striking. DAPI stained images (B and D) are matched with the FITC-phalloidin images. Fluorescein isothiocyanate (FITC) stained microgrooved gelatin gels. XZ-reconstructions of fresh microgrooved gelatin (E) and 24hrs of submerged culture followed by 48hrs of air-liquid-interface (F) microgrooved gelatin; magnification 40x; 29.70um complete stack made up of 18 slices (1.65um per slice). Scale bars are 50um.

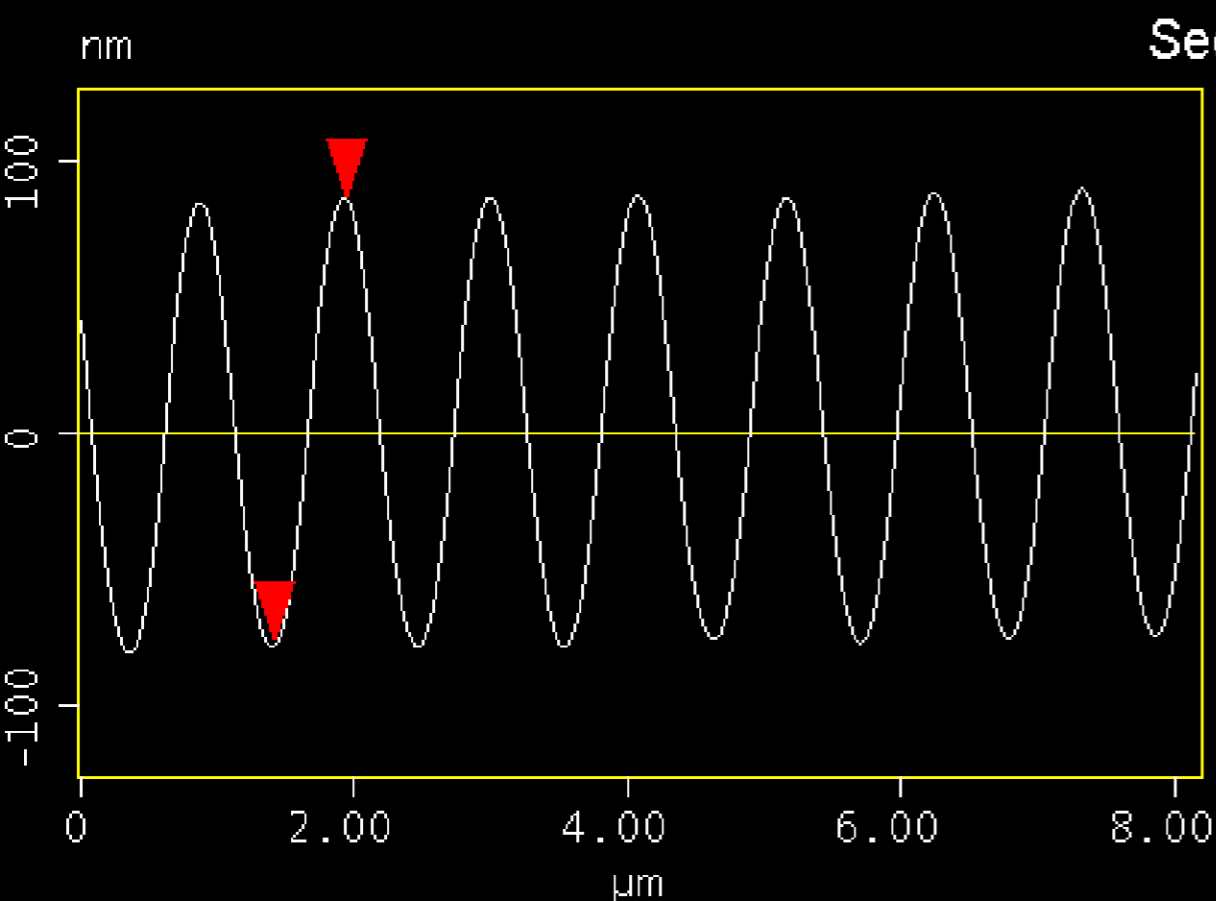
Supplemental Figure 8. BEAS2B cell alignment after groove (3.14um deep) system treatments. Microgroove systems with 3.14um depth were seeded with BEAS2B for 24 hours of submerged culture, followed by 48 hours of ALI culture (A), then were trypsinized to lift the cells off the grooves (B), then aspirated to remove all the BEAS2B (C), and finally seeded with new BEAS2B for 48 hours of submerged culture (D) and imaged for alignment (E). BEAS2B were also seeded on microgrooved gelatin constructs after 48hrs of ALI culture without cells and imaged (F). Angular histograms corresponding to the above substrate condition quantify cell alignment on substrates, where the red line indicates the direction of the grooves. Scale bar A-D is 100um; E –F is 50um.

24hr SC + 72h ALI collagen coated transwell

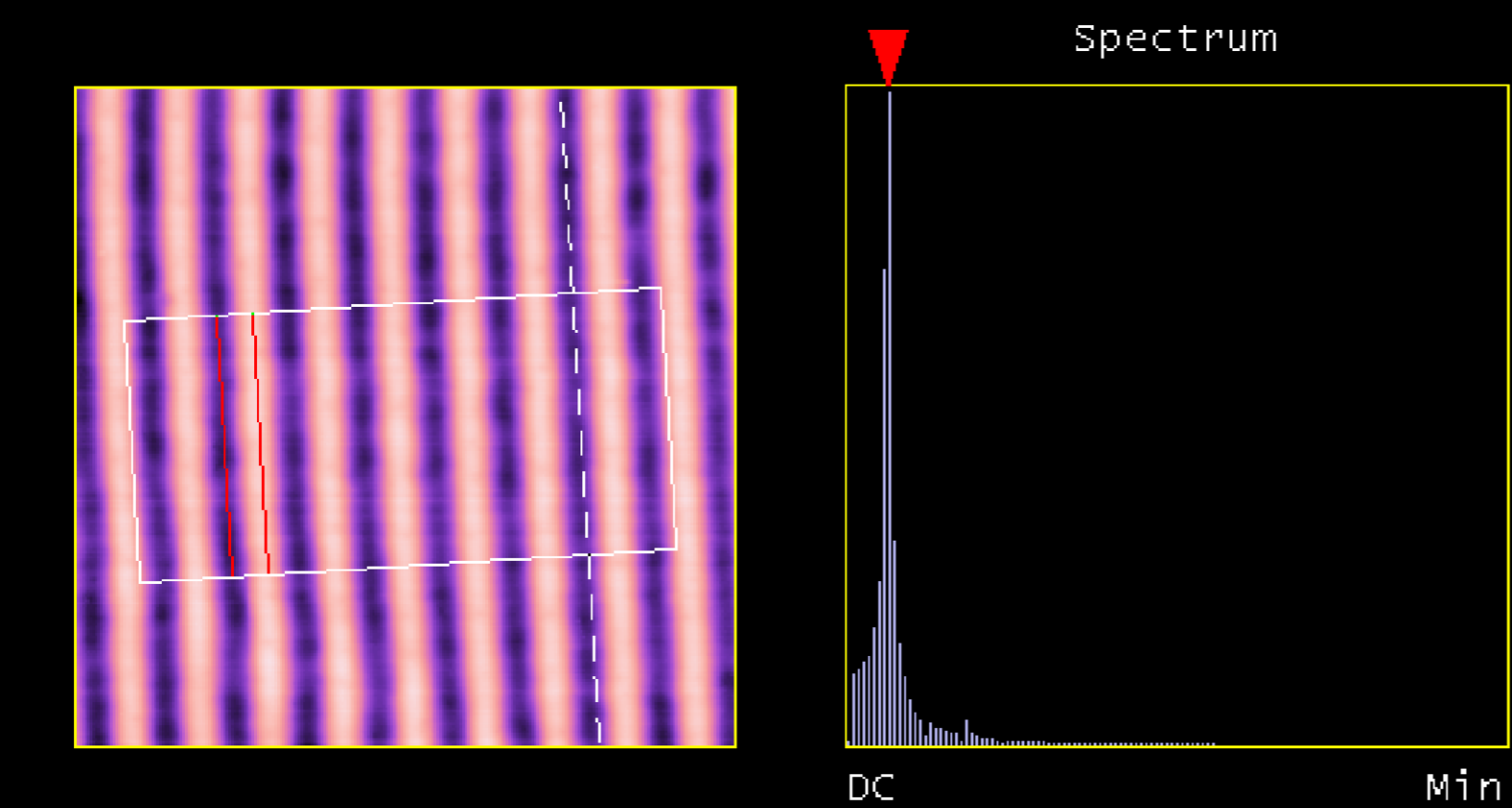
24hr SC + 72h ALI flat gelatin

24hr SC + 72h ALI deep grooved gelatin





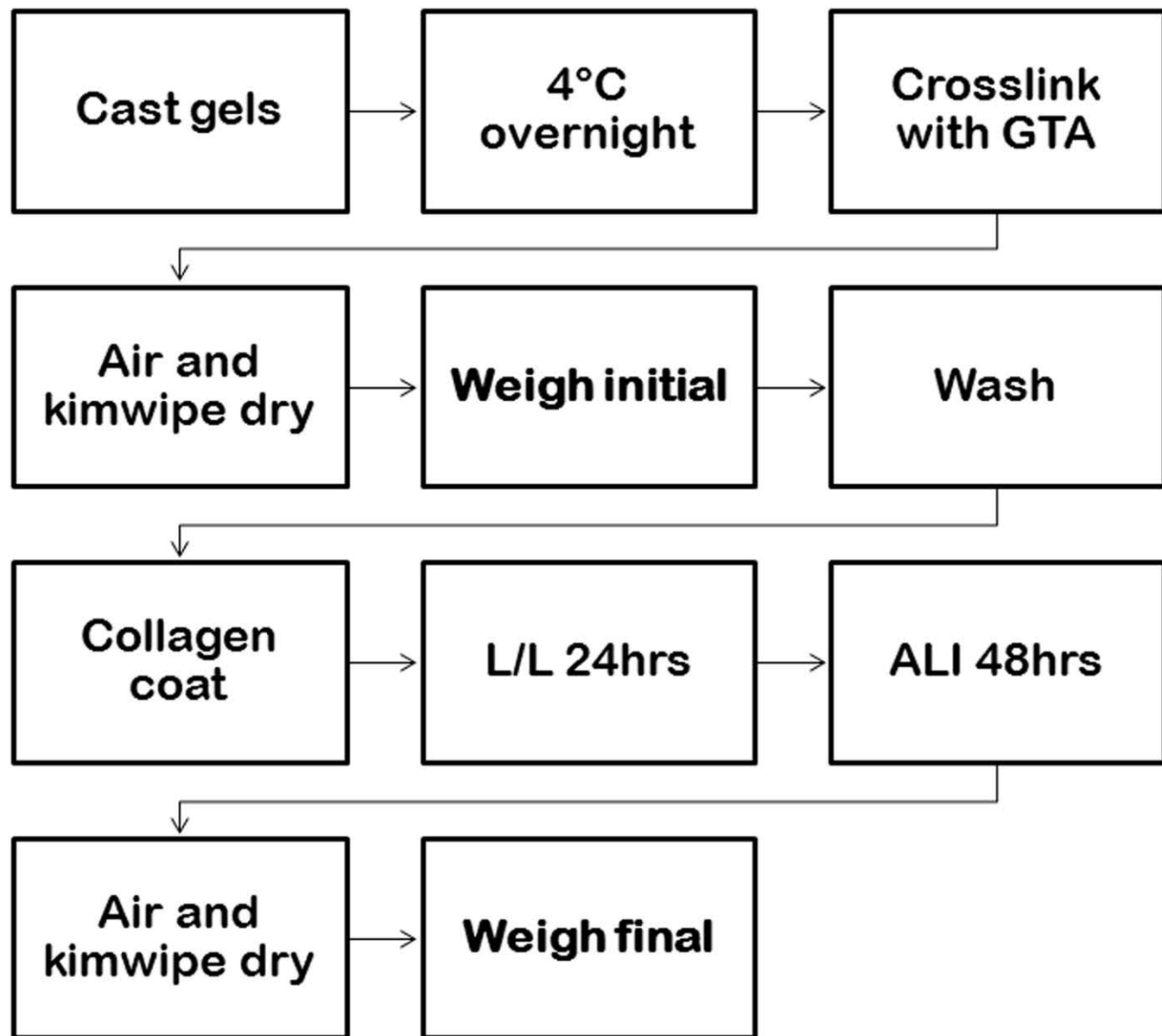
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RMS	60.124 nm
lc	1.023 μ m
Ra(lc)	3.082 nm
Rmax	41.338 nm
Rz	30.941 nm
Rz Cnt	4
Radius	530.96 nm
Sigma	21.328 nm



Surface distance	559.29 nm
Horiz distance(L)	527.34 nm
Vert distance	162.00 nm
Angle	17.077 °
Surface distance	
Horiz distance	
Vert distance	
Angle	
Surface distance	
Horiz distance	
Vert distance	
Angle	
Spectral period	1.023 μ m
Spectral freq	0.978 / μ m
Spectral RMS amp	43.254 nm

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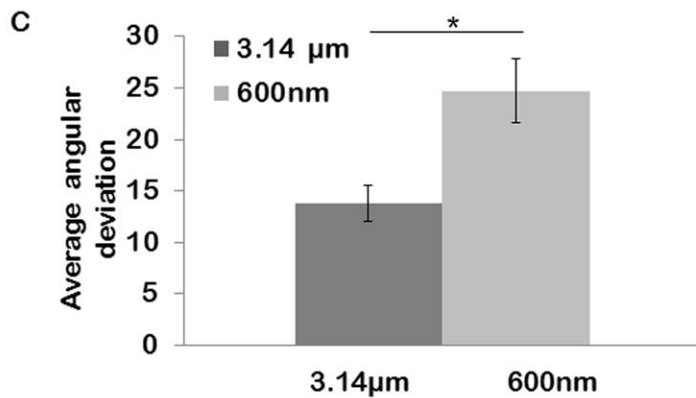
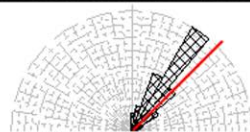
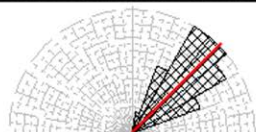
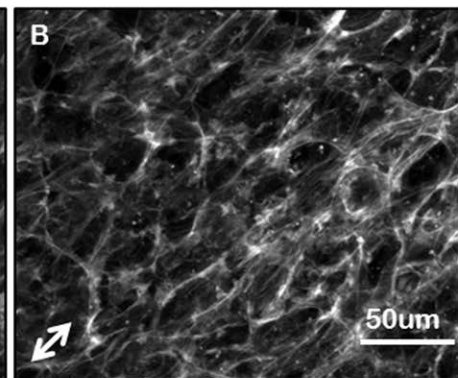
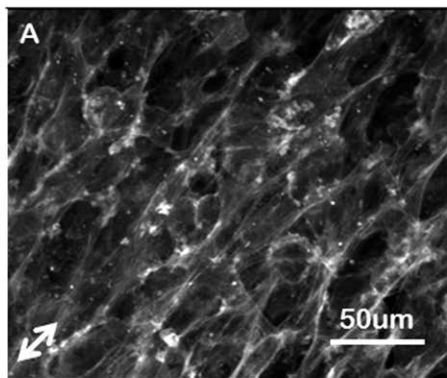
A



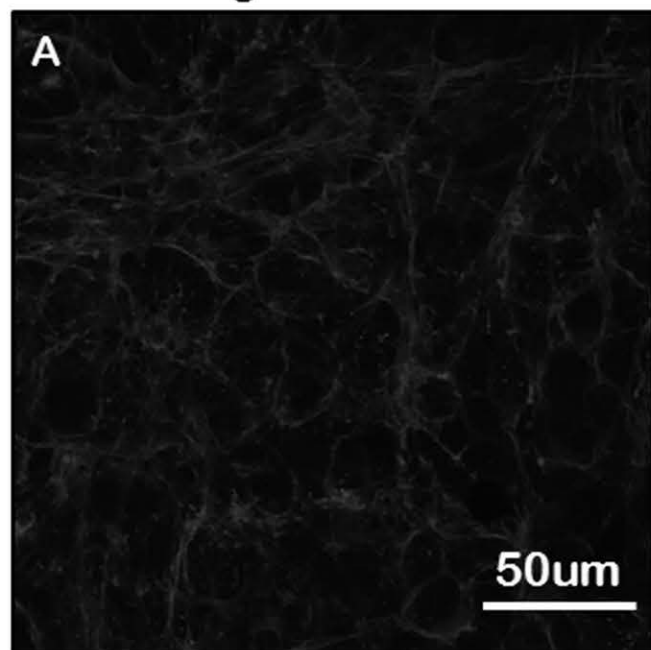
B

Gelatin gel concentration (% w/w)	[GTA] (% v/v)	0.1	0.25	0.5
1.5			$-4.158 \pm 0.181\%$	$-4.763 \pm 0.465\%$
2			$-5.771 \pm 0.702\%$	$-5.392 \pm 0.822\%$
3		$-6.891 \pm 1.05\%$	$-5.907 \pm 0.706\%$	$-7.468 \pm 0.537\%$
5		$-4.247 \pm 0.202\%$	$-7.127 \pm 0.435\%$	$-7.129 \pm 0.607\%$
10		$0.225 \pm 3.26\%$	$-5.226 \pm 0.090\%$	$-5.578 \pm 0.129\%$

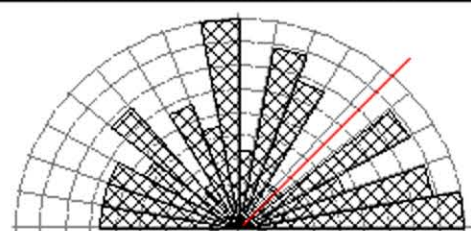
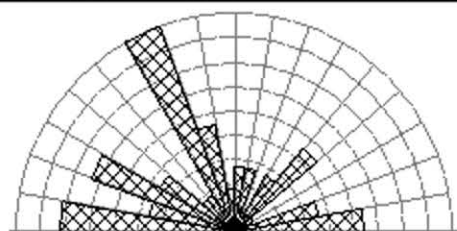
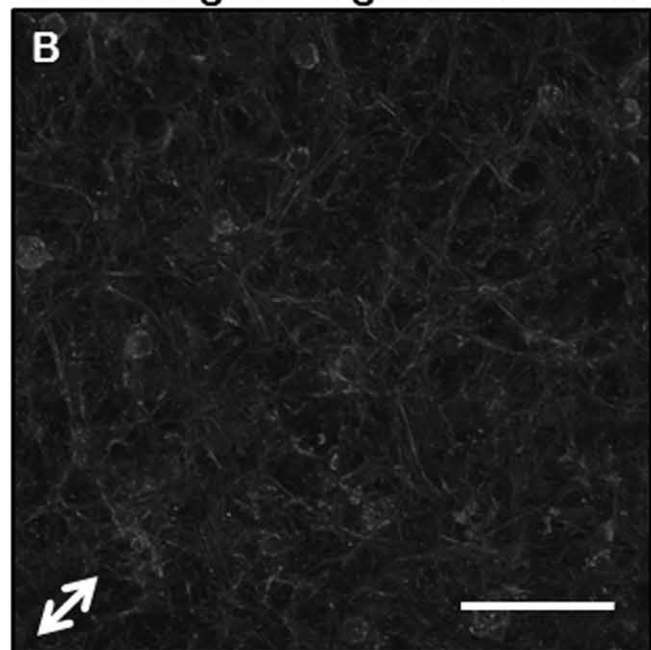
F-Actin



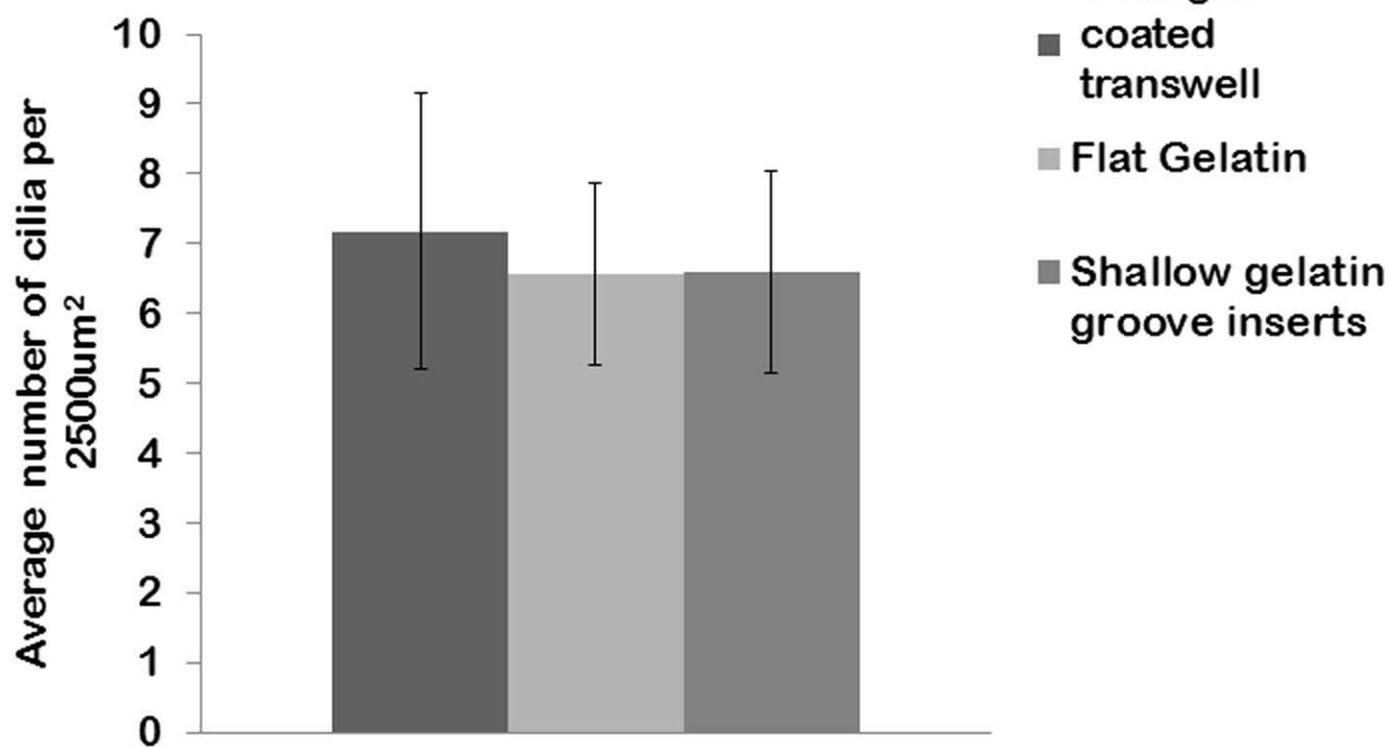
Flat gelatin inserts

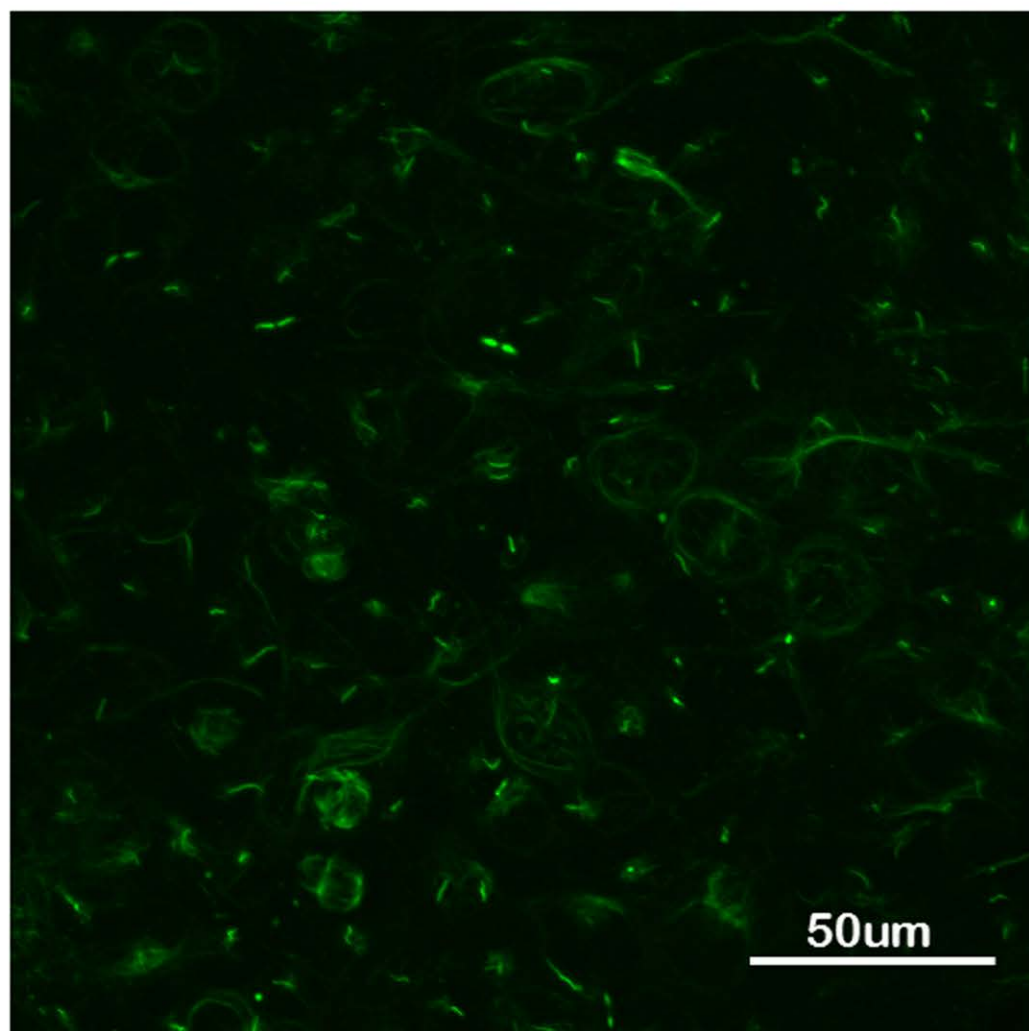


Shallow groove gelatin inserts



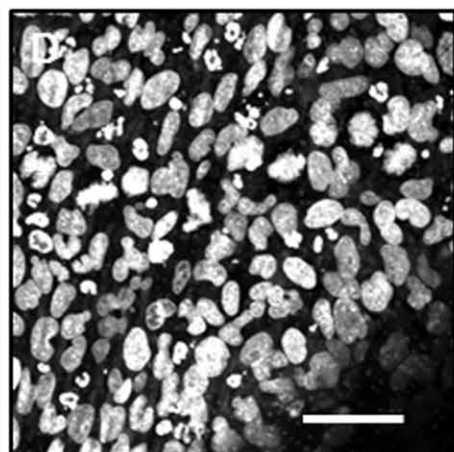
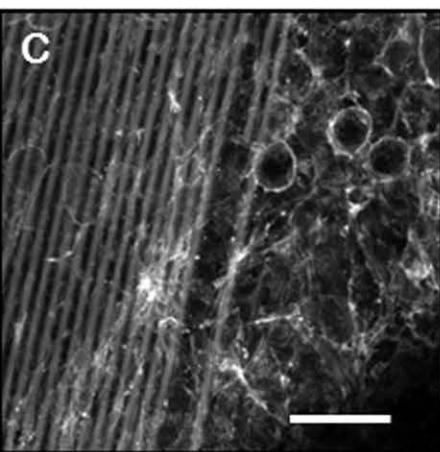
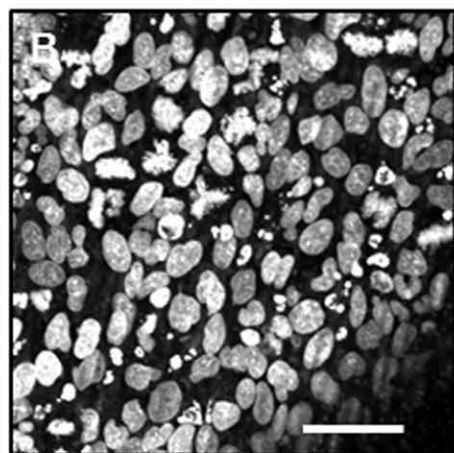
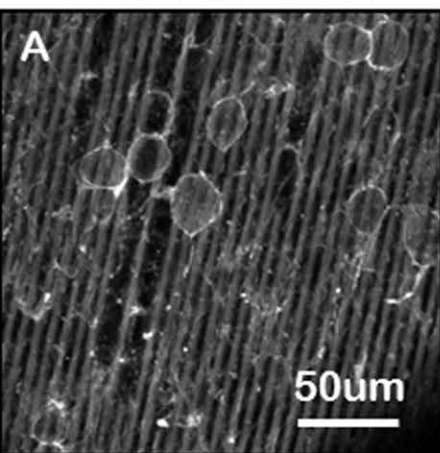
C





FITC-Phalloidin

DAPI



NG gelatin

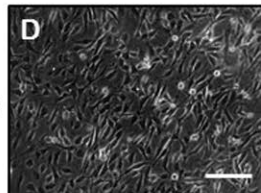
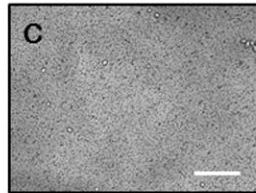
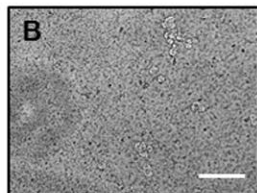
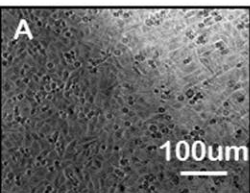


24hr SC+ 48hr
ALI culture of
BEAS2B

Trypsinize

Aspirate

Seed new
BEAS2B for
48hr



24hrs SC+48hrs ALI – Trypsinize – 48hrs SC

Acellular 48hrs ALI + 48hrs SC

NG gelatin

