# **Supporting Information**

# Nanopillar Array on Black Titanium Prepared by Reactive Ion Etching Augments Cardiomyogenic Commitment of Stem Cells

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### Materials and methods

### **Protein adsorption studies**

Two protein solutions of known concentration namely 10% fetal bovine serum and 50  $\mu$ g/ml of Fibronectin were used to assess the protein adsorption on Ti samples. Both c-Ti and b-Ti disks were incubated at 37°C in these solutions individually using diluent buffer as the control. After 24 h, the excess protein solution was removed and samples were gently washed to remove loosely attached left over protein. To quantify the actual amount of protein interacting with the surfaces, the samples were sonicated in PBS for 30 min and BCA assay was performed for protein quantification. The amount of adsorbed protein was calculated in  $\mu$ g per unit surface area of the titanium disk used (Table S1).

### Fabrication and Characterization of Si pillar

Pillars on silicon (Si) were prepared by UV lithography followed by reactive ion etching (RIE) technique<sup>1</sup>. Briefly, the wafer is subjected to -Piranha cleaning for 10 min and then 5  $\mu$ m thick layer of photoresist (AZ nLOF 2070) was spin-coated onto the silicon wafers. After a soft baking process at 65° C for 1 min, photo-masks with geometry of 3  $\mu$ m diameter and 3  $\mu$ m spacing between each pillar was used. The Si substrates were exposed to UV light for 8 seconds in vacuum contact mode using MJB4 Mask Aligner (Suss Microtec). After exposure to UV light, the samples were baked at 95° C for 1 min. The resist was developed using MIF 726 (MicroChemicals). Finally, RIE was done with a gas mixture of C<sub>4</sub>F<sub>8</sub> (90 sccm) and SF<sub>6</sub> (50 sccm), chamber pressure of 9 mTorr and ICP power of 1200 W using PlasmaLab system 100 ICP 180 (Oxford Instruments) to etch the silicon and obtain the micropillars. The remaining resist was removed using 70% ethanol and exposure to UV for 30 min. Surface morphology of the Si pillars and Si control surfaces were studied using scanning electron

microscope (SEM, Zeiss Gemini with MonoCL). The samples were dried in desiccator and mounted on an aluminum stub and sputter coated with gold before imaging<sup>2</sup>.

### Evaluation of stem cell response on Si surfaces

Cardiomyogenic lineage commitment was assessed by immunostaining for cardiac specific marker proteins namely Connexin43 (Cx43) (Thermo Scientific, USA). Primary antibodies for Cx43 was raised in mice. Cells were fixed at day 7 with 4% (m/v) paraformaldehyde in PBS for 15 min. The cell membrane was then permeabilized using 0.2% Triton-X solution. Blocking was done using 5% BSA at 25° C for 45 min. The cells were incubated with primary antibody at dilution of 1:200 overnight at 4° C. The primary antibodies were removed and washed thoroughly with PBS. This was followed by incubation with goat antimouse Alexa Flour 488 conjugated secondary antibody for Cx43 for 2 h at 25° C at a dilution of 1:500. Further the actin filaments in cells were stained with Alexa Flour 546 Phalloidin (Thermo Scientific, USA) for 30 mins. The nuclei were then stained with 0.5  $\mu$ g/ml of DAPI for 30 s at 25° C. The samples were mounted on a glass coverslip using ProLong Diamond antifade mountant (Thermo Fisher Scientific) and imaged using confocal microscope (LSM 710, Zeiss, USA).

### References

- 1. J. Hasan, S. Raj, L. Yadav and K. Chatterjee, *RSC Advances*, 2015, **5**, 44953-44959.
- 2. L. D. Ghosh, V. Ravi, P. Sanpui, N. R. Sundaresan and K. Chatterjee, *Colloids and surfaces*. *B*, *Biointerfaces*, 2017, **151**, 178-188.

	Samples	Absorbed protein (µg/cm²)
No protein	c-Ti	0
	b-Ti	0
10% FBS	c-Ti	0.02±0.01
	b-Ti	0.03±0.01
Fibronectin (50µg/ml)	c-Ti	0.03±0.01
	b-Ti	0.02±0.01

# Table S1: Comparison of protein adsorption on Ti surfaces

### Figure S1



Figure S1: Photograph of the Titanium surfaces (a) smooth control surface; and (b) etched nanopillared titanium surfaces.

## Figure S2



Figure S2: Scanning electron micrographs of the fabricated nanopillars on the surface of titanium. Scale bar =1  $\mu$ m. (a) Topography of the fabricated nanopillars; (b) cross sectional view of the nanopillars fabricated on titanium surface by reactive ion etching.

### Figure S3



Figure S3: Scanning electron micrographs of hMSCs cultured on titanium surfaces at day 1 and day 7. Scale bar = 1  $\mu$ m. The insets show the closer view of cell interactions with the smooth control and nanopillared titanium topography. Scale bar = 200 nm for the insets.

### **Figure S4**



Figure S4: Confocal micrographs of Actinin (green) stained hMSC at day 7. (a) hMSCs cultured on TCPS surface exposed to Aza for 20 h after seeding; (b) hMSCs cultured on b-Ti surface without Aza treatment post seeding; (c) hMSCs cultured on b-Ti with exposure to Aza for 20 h after seeding. Nucleus stained with DAPI appears blue in the images. Scale bar =  $25 \mu$ m. Highest expression of cardiomyogenic marker, Actinin, is seen in (c).

Figure S5



Figure S5: Si with pillars. (a, b) Scanning electron micrographs of control Silicon (Si Control) and micropillar Silicon (Si micropillar) surfaces. Scale bar= 20  $\mu$ m; (c-d) Scanning electron micrographs of hMSC cultured on Si Control and Si micropillar respectively. Scale bar= 100  $\mu$ m; (e-f) Confocal micrographs of hMSC cultured on Si surfaces and stained for cardiac marker Connexin43 (Cx43 in green), Actin (red) and DAPI stained nucleus (blue). Scale bar= 25  $\mu$ m. Presence of Si micropillar may be noted in (f) below the cells.

### Supporting Videos V1 and V2 are available online