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Supporting Information

Self-assembled binary colloidal crystal layers as cell culture substrates

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Equation S1. Volume calculation of colloidal suspensions needed for a BCC layer (one layer of the large particles is assumed).

$$V = \frac{Ap \times \rho \times Vp \times 0.95}{Ao \times w\%} \quad \textbf{(1)}$$

V = the final volume of the particle solution (μL). Ap = Projection area of particle (cm^2). ρ = density of particle (g/cm^3). Ao = inside area of O-ring (cm^2). w% = weight percentage of particle solution (g/cm^3). Vp = volume of particle (cm^3).

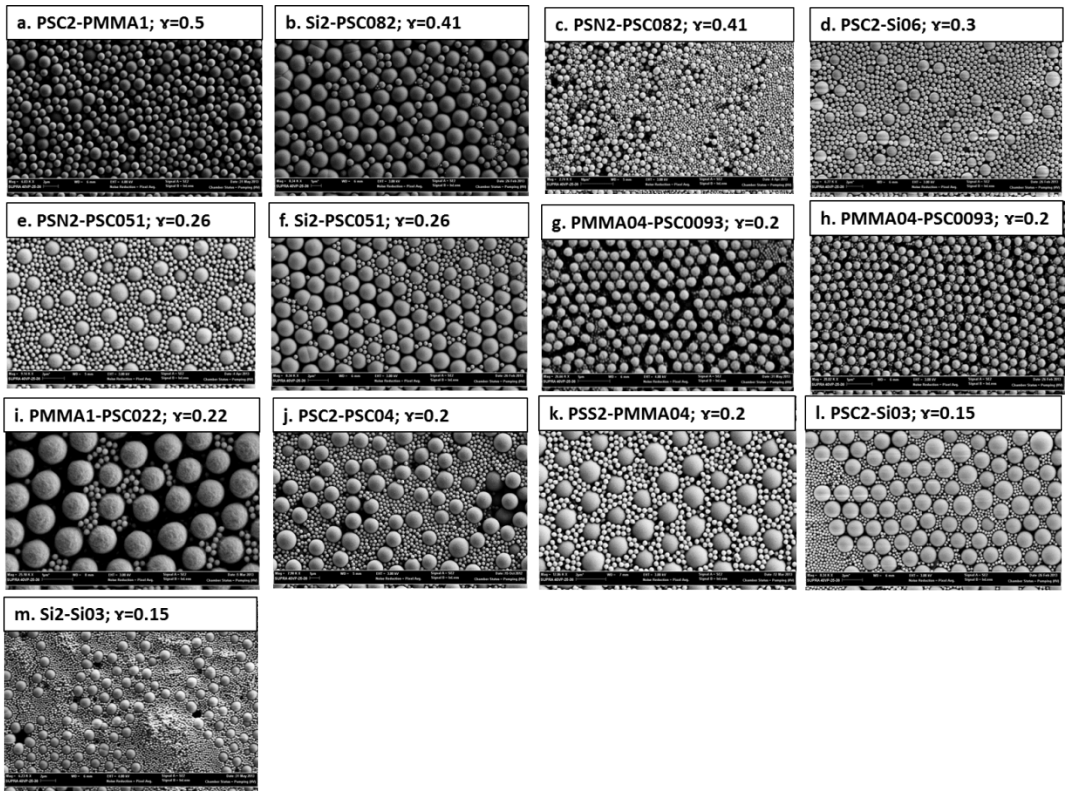
Table S1. A list of the different size combination in critical window of BCCs. All selected particles are stable particles with zeta potentials > |±30 mV| and all combination have size ratios (small/large) < 0.2. It should be noted that BCC structure also can be found occasionally for size ratios > 0.2, but with small surface coverage. Surface coverage and BCC structure coverage were classified as high (> 70%), medium (70%-40%), and low (< 40%). Combinations with both high surface coverage and high BCC structure coverage were eventually selected as cell culture substrates. To be noted, combination outside the window doesn't show in this table.

No.	Large Particle	µm	Small Particle	µm	Size ratio	Surface Coverage	BCC structure
1	Si	5	PMMA	1	0.200	high	no
2	Si	5	PMMA	0.4	0.080	high	high
3	Si	5	PSC	1	0.200	high	medium
4	Si	5	PSC	0.82	0.164	high	low
5	Si	5	PSC	0.75	0.150	high	low
6	Si	5	PSC	0.51	0.102	high	high
7	Si	5	PSC	0.4	0.080	high	high
8	Si	5	PSC	0.22	0.044	high	high
9	Si	5	PSC	0.093	0.019	high	high
10	Si	5	PSC	0.06	0.012	high	high
11	Si	5	PSC	0.024	0.005	high	low
12	Si	5	Si	0.6	0.120	high	no
13	Si	5	Si	0.31	0.062	high	low
14	PSC	3	PSC	0.51	0.170	high	low
15	PSC	3	PMMA	0.4	0.133	high	medium
16	PSC	3	PSC	0.4	0.133	high	medium
17	PSC	3	PSC	0.22	0.073	high	medium
18	PSC	3	PSC	0.093	0.031	high	medium
19	PSC	3	PSC	0.06	0.020	medium	low
20	PSC	3	PSC	0.024	0.008	medium	low
21	PSC	3	Si	0.6	0.200	high	no
22	PSC	3	Si	0.31	0.103	high	low
23	PSC	2	PMMA	0.4	0.200	high	medium
24	PSC	2	PSC	0.4	0.200	medium	low
25	PSC	2	PSC	0.22	0.110	high	low
26	PSC	2	PSC	0.093	0.047	high	low
27	PSC	2	PSC	0.06	0.030	medium	low
28	PSC	2	Si	0.3	0.150	high	low
29	PSC	2	PSC	0.024	0.012	high	low
30	PSS	2	PMMA	0.4	0.200	high	no
31	PSS	2	PSC	0.4	0.200	high	low
32	PSS	2	PSC	0.22	0.110	high	low
33	PSS	2	PSC	0.093	0.047	high	low
34	PSS	2	PSC	0.06	0.030	high	medium
35	PSS	2	PSC	0.024	0.012	high	no
36	PSS	2	Si	0.3	0.150	high	no
37	Si	2	PSC	0.4	0.200	high	low
38	Si	2	PMMA	0.4	0.200	high	low
39	Si	2	PSC	0.22	0.110	high	high
40	Si	2	PSC	0.093	0.047	high	high
41	Si	2	PSC	0.06	0.030	high	medium
42	Si	2	PSC	0.024	0.012	high	medium
43	Si	2	Si	0.3	0.150	high	low
44	PMMA	1	PSC	0.093	0.093	medium	low
45	PMMA	1	PSC	0.06	0.060	high	no
46	PMMA	1	PSC	0.024	0.024	medium	no
47	PSC	1	PSC	0.093	0.093	high	low
48	PSC	1	PSC	0.06	0.060	high	low
49	PSC	1	PSC	0.024	0.024	low	low

Table S2. A list of 24 colloidal particles. The particle size, supplier and catalogue number of each particle are listed. PSC: carboxylated polystyrene; PSN: aminated polystyrene; PMMA: polymethyl methacrylate; SiC: carboxylated silicon; SiN: aminated polystyrene; PSS: sulphated polystyrene.

#	Colloid	Diameter	Supplier	Cat. #
1	PSC 3	3 µm	Polyscience	19819-1
2	PSC 2	2 µm	Invitrogen	C37278
3	PSC 1	1 µm	Polyscience	19819-1
4	PSC 0.8	0.82 µm	Bang Lab	PCO3N/10129
5	PSC 0.75	0.75 µm	Polyscience	19819-1
6	PSC 0.5	0.51 µm	Bang Lab	PCO3N/11085
7	PSC 0.4	0.4 µm	Invitrogen	C37268
8	PSC 0.2	0.22 µm	Bang Lab	PCO2N
9	PSC 0.093	0.093 µm	Bang Lab	PCO2N/7576
10	PSC 0.06	0.06 µm	Invitrogen	C37263
11	PSC 0.024	0.024 µm	Bang Lab	PCO2N/9928
12	PSN 2	2 µm	Invitrogen	A37366
13	PSN 0.4	0.4 µm	Invitrogen	A37358
14	PSN 0.2	0.18um	Bang Lab	PA02N/9328
15	PSN 0.06	0.06 µm	Invitrogen	A37353
16	PSS 2	2 µm	Polyscience	19405-10
17	PMMA 1	1.1 µm	Bang Lab	PP04N/10710
18	PMMA 0.4	0.4 µm	Bang Lab	PP02N/9861
19	Si 5	5 µm	Bang Lab	SS06N
20	Si 2	2.01um	Bang Lab	SS04N/7829
21	Si 0.6	0.6 µm	Bang Lab	SSO3N/5688
22	Si 0.3	0.31um	Bang Lab	SS02N/9959
23	SiC 2	2 µm	Bang Lab	SC04N/9395
24	SiN 1	1 µm	Bang Lab	SA04N/9083

A


a. PSC2-PMMA1; ϒ=0.5
b. Si2-PSC082; ϒ=0.41
c. PSN2-PSC082; ϒ=0.41
d. PSC2-Si06; ϒ=0.3
e. PSN2-PSC051; ϒ=0.26
f. Si2-PSC051; ϒ=0.26
g. PMMA04-PSC0093; ϒ=0.2
h. PMMA04-PSC0093; ϒ=0.2
i. PMMA1-PSC022; ϒ=0.22
j. PSC2-PSC04; ϒ=0.2
k. PSS2-PMMA04; ϒ=0.2
l. PSC2-Si03; ϒ=0.15
m. Si2-Si03; ϒ=0.15

B

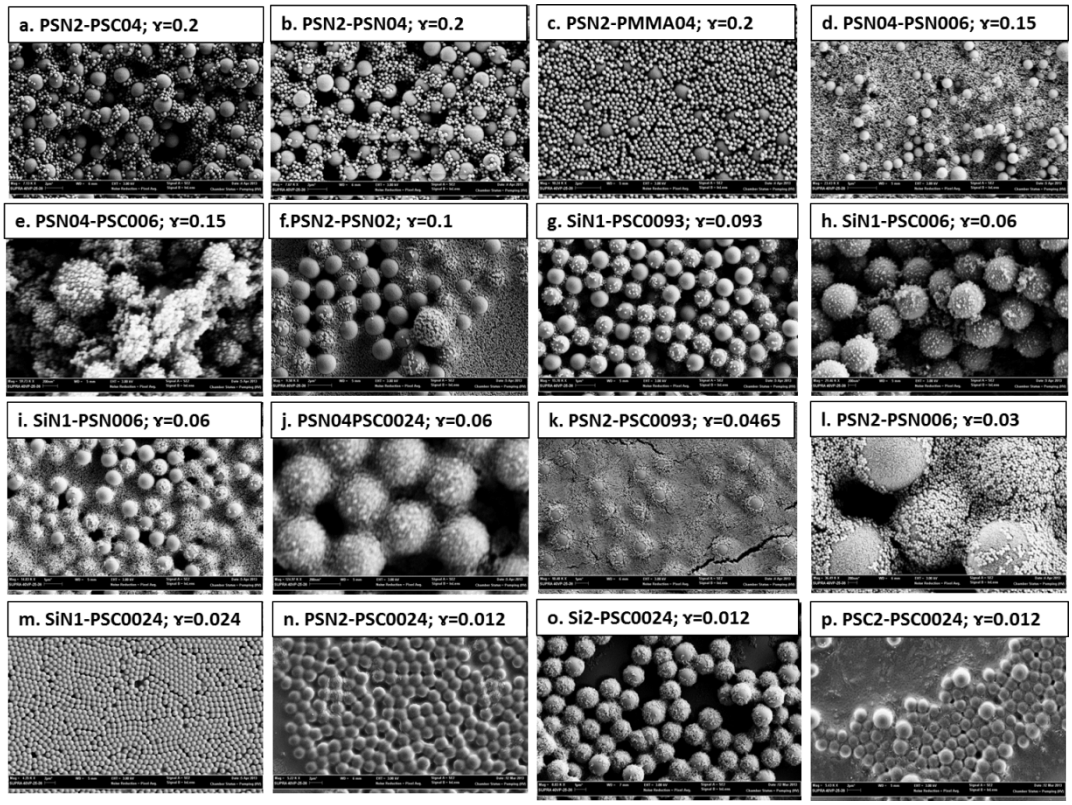

a. PSN2-PSC04; ϒ=0.2
b. PSN2-PSN04; ϒ=0.2
c. PSN2-PMMA04; ϒ=0.2
d. PSN04-PSN006; ϒ=0.15
e. PSN04-PSC006; ϒ=0.15
f.PSN2-PSN02; ϒ=0.1
g. SiN1-PSC0093; ϒ=0.093
h. SiN1-PSC006; ϒ=0.06
i. SiN1-PSN006; ϒ=0.06
j. PSN04PSC0024; ϒ=0.06
k. PSN2-PSC0093; ϒ=0.0465
l. PSN2-PSN006; ϒ=0.03
m. SiN1-PSC0024; ϒ=0.024
n. PSN2-PSC0024; ϒ=0.012
o. Si2-PSC0024; ϒ=0.012
p. PSC2-PSC0024; ϒ=0.012

Figure S1. Binary colloidal crystal assemblies with (A) disordered structures and (B) small particle adsorption onto larger particles. Selected images showed different size ratios (γ) ranging 0.15-0.5 for disordered structures and 0.012-0.2 for particle adsorption. In each case, the sample description indicates the large particle/small particle type and size (micron) used and also provides the particle size ratio (γ). PSC: carboxylated polystyrene; PSN: aminated polystyrene; PSS: sulphated polystyrene; PMMA: polymethyl methacrylate; SiC: carboxylated silica; SiN: aminated silica.

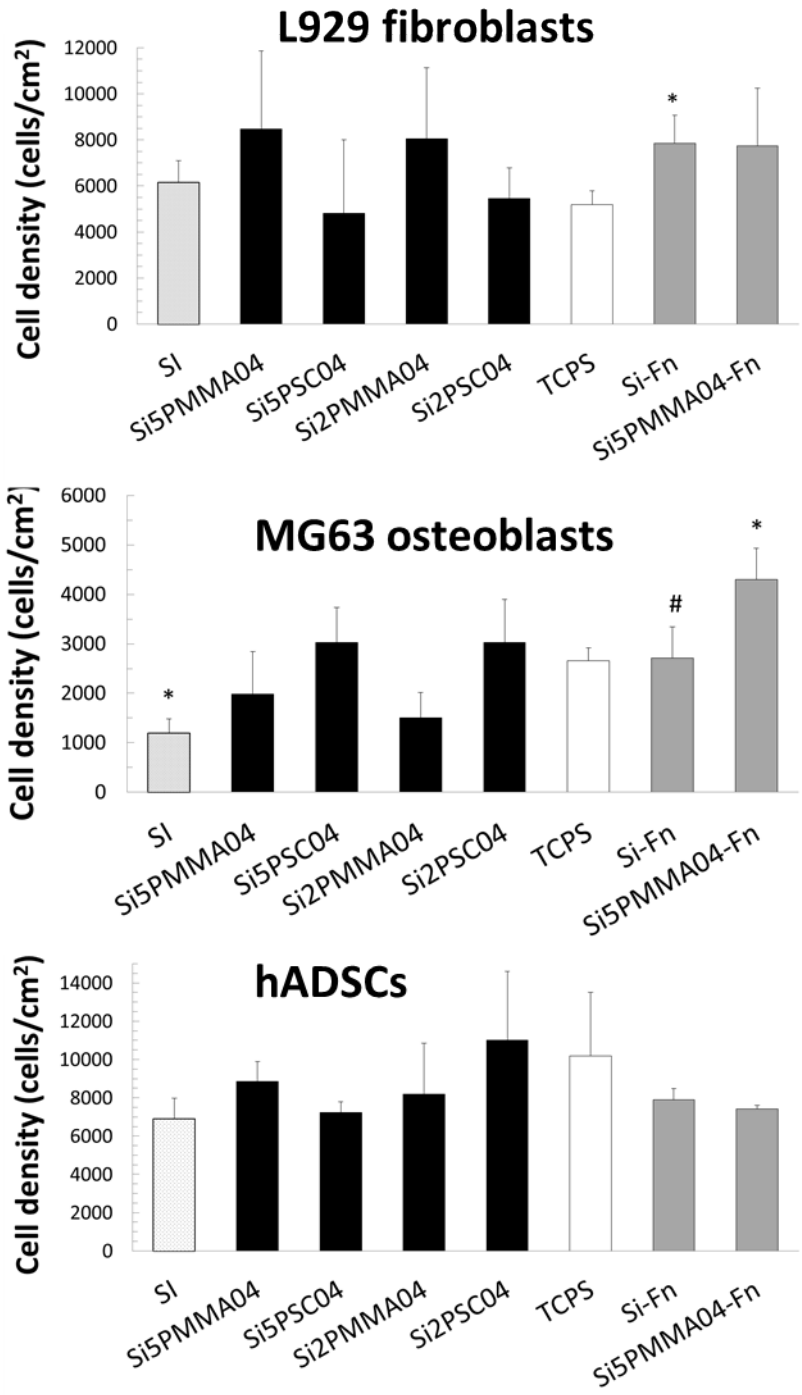


Figure S2. Cell attachment density on surfaces after washing. The density of fibroblasts and hADSCs across surfaces was similar, even after FN coating, whilst the density of osteoblasts was dependent on the topography and chemistry of the substrates used. Values = mean ± standard deviation (n = 5). * indicates a significant difference versus TCPS. One, two and three symbols indicate $p < 0.05$, 0.01, and 0.001, respectively.

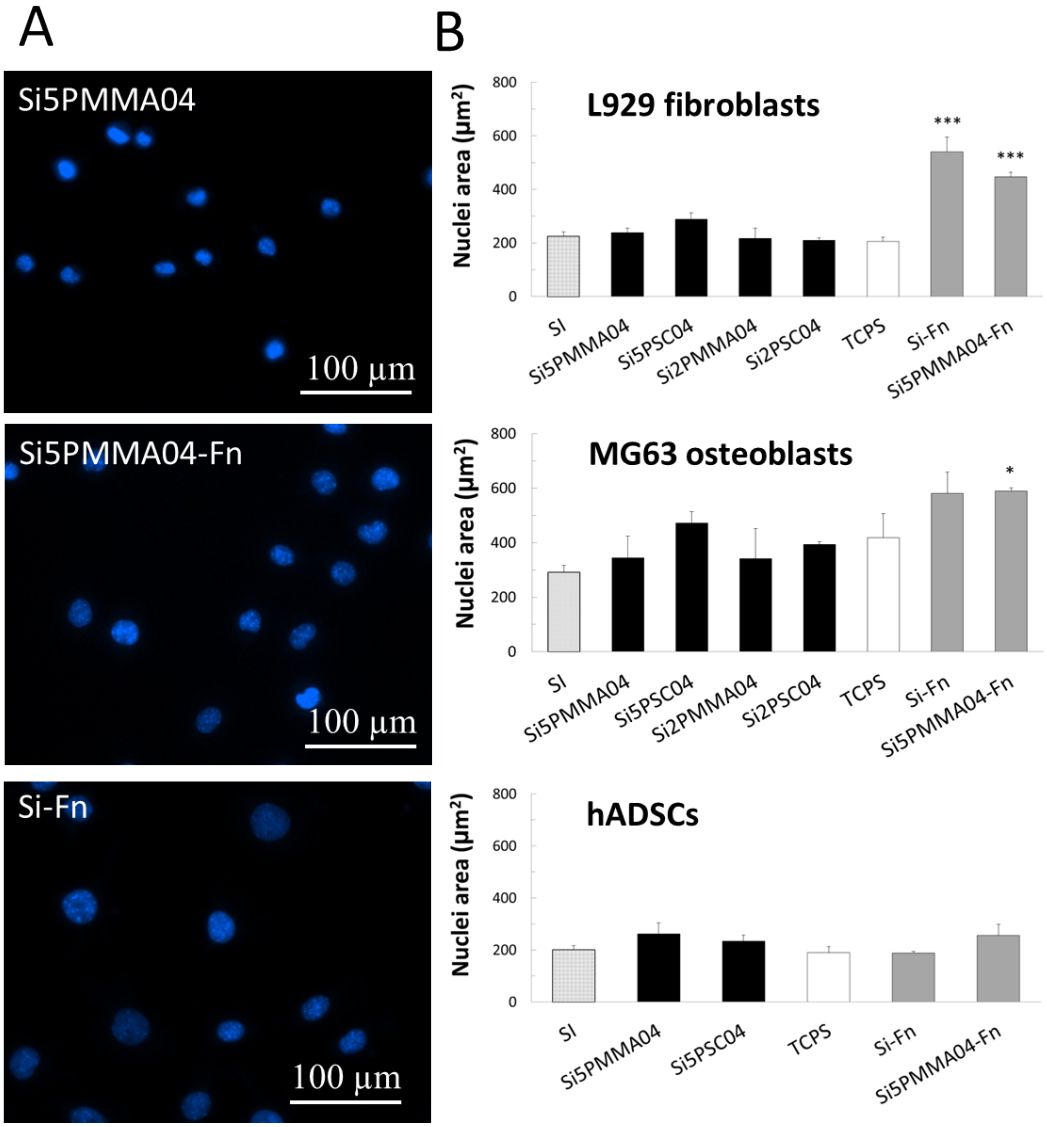


Figure S3. The projection area of cell nuclei. (A) Cell nuclei of fibroblasts on different surfaces. The projection area of cell nuclei increased when surfaces were coated with Fn. (B) Statistically, no significant difference was observed between the projection area of cell nuclei of three different cell types on different surfaces before Fn coating. However, after FN coating, the nucleus area of fibroblasts was increased in some cases. Values = mean±standard deviation (81-422). * indicates that there is a significant difference versus TCPS. One, two and three symbols indicate $p < 0.05$, 0.01, and 0.001, respectively.

A. 2 μm PS-COOH

B. 2 μm PS-COOH/400nm PMMA

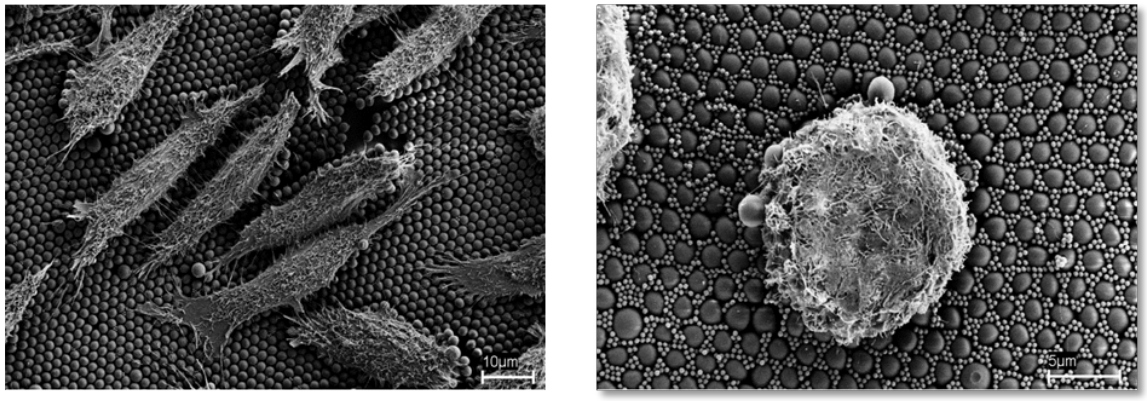


Figure S4. The morphology of L929 fibroblast attached to (A) single (SCC) and (B) binary colloidal crystal (BCC) monolayers. Cells were elongated on SSC comprised of 2 μm PS-COOH, while they are rounded on BCC comprised of 2 μm PS-COOH and 400 nm PMMA. Scale bar = 10 μm in A and 5 μm in B.