Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

# Utilization of star-shaped polymer architecture in the creation of high-density polymer brush coatings for the prevention of platelet and bacteria adhesion

Masayasu Totani, Tsuyoshi Ando*, Kayo Terada, Takaya Terashima, Ill Yong Kim,

Chikara Ohtsuki, Chuanwu Xi, Kenichi Kuroda*, Masao Tanihara

## 1. Syntheses of star polymers

### *1-1. Syntheses of homoarm star polymers*

**star-PMMA:** Synthesis of PMMA star polymer (star-PMMA) was carried out by syringe technique under argon in baked glass flask equipped with a three-way stopcock. Precursor PMMA (14.9 g, $M_n$ = 8,300, $M_w/M_n$ = 1.25, 1.80 mmol), EGDMA (3.40 mL, 18.0 mmol), toluene (21.7 mL), and a solution of $Ru(Ind)Cl(PPh_3)_2$ in toluene (2.06 mM, 175 mL, 0.361 mmol), *n*-$Bu_3N$ (0.858 mL, 3.61 mmol) were sequentially added into a 500 mL baked flask. Immediately after mixing, polymer solution was separated in three aliquots into the 100 mL flask at room temperature under argon. The mixtures were degassed by three freeze-pump-thaw cycles, and placed in an oil bath at 80 ºC. After 56 h, the reaction was terminated by cooling the mixtures in an ice bath. Solvent was removed under reduced pressure. The residue was dissolved in toluene (20wt%), and then the quintuple volume of methanol was poured into the polymer solution to remove unreacted PMMA. The PMMA star was then dissolved in toluene and passed through silica gel and alumina columns to remove ruthenium complex. The solvent was concentrated under reduced pressure, and then polymer was precipitated with hexane. The precipitate was dried overnight under vacuum at room temperature to give final products as a white powder. $M_w$ = 209,000, MWD =1.15 (SEC-MALLS). $^1H$ NMR (500.16 MHz, $CDCl_3$, $Si(CH_3)_4 = 0$ ppm): $\delta$ (ppm) = 3.70-3.40 (-$OCH_3$), 2.10-1.30 (-$CH_2$-), 1.30-0.60 (-$CH_3$).

**star-PHEMA:** In a 100 mL round bottomed flask, PTMSOEMA (10.8 g, 0.668 mmol, $M_n$ = 16,200, $M_w/M_n$ = 1.30), $Ru(Ind)Cl(PPh_3)_2$ (0.156 g, 0.134 mmol), toluene (67.5 mL), *n*-$Bu_3N$

(0.318 mL, 1.34 mmol), EGDMA (1.26 mL, 6.68 mmol) were added sequentially in this order at room temperature under argon. Immediately after the degassed by three freeze-pump-thaw cycles, the mixture was placed in an oil bath at 80 ºC. After 52 h, the reaction was terminated by cooling the mixture in an ice bath. The obtained star polymer was dissolved in acetone (20 wt%), and then added into 0.2 times volume with water to remove unreacted PTMSOEMA. Ru-complex was removed by silica-gel and alumina column chromatographies eluted with toluene. TMS protecting group was removed by the addition of a small volume of 1.5 *N* HClaq. to the polymer solution in THF. The resulting solution was poured into water to precipitate a star polymer. It was separated by suction filtration and dried under vacuum overnight at room temperature**:** $M_w$ = 286,300, $M_w/M_n$ =1.25 (SEC-MALLS). $^1$H NMR (500.16 MHz, $CD_3OD$, $Si(CH_3)_4$ = 0 ppm): δ (ppm) = 4.16-3.85 ($-CH_2-CH_2-OH$), 3.85-3.59 ($-CH_2-CH_2-OH$), 2.19-1.42 ($-CH_2-$), 1.42-0.67 ($-CH_3$).

### *1-2. PHEMA / PMMA heteroarm star polymers*

**star-H47M53**: All experimental procedures for synthesis of a series of heteroarm star polymers consisting of PHEMA and PMMA were similar to star PMMA synthesis. In a 100 mL round bottomed flask, PTMSOEMA (5.41 g, 0.334 mmol, $M_n$ = 16,200, $M_w/M_n$ = 1.30), PMMA (2.77 g, 0.334 mmol, $M_n$ = 8,300, $M_w/M_n$ = 1.25), $Ru(Ind)Cl(PPh_3)_2$ (0.156 g, 0.134 mmol), toluene (70.1 mL), *n*-$Bu_3N$ (0.32 mL, 1.34 mmol), EGDMA (1.26 mL, 6.68 mmol) were added sequentially in this order at room temperature under argon. Immediately after the degassed by three freeze-pump-thaw cycles, the mixture was placed in an oil bath at 80 ºC. After 52 h, the reaction was terminated by cooling the mixture in an ice bath. The obtained star polymer was dissolved in toluene (20wt%), quintuple volume of hexane was added into the polymer solution to remove unreacted PTMSOEMA, and then the polymer was dried under reduced pressure. The polymer was dissolved in acetone (20wt%), and then added into 0.2 times volume with water to remove unreacted PMMA. Ru-complex was removed by silica-gel and alumina column chromatographies eluted with toluene. TMS protecting group was removed by the addition of a small volume of 1.5 *N* HClaq. to the polymer solution in ethanol/acetone (1/1 v/v). The resulting solution was poured into hexane to precipitate a star polymer. It was separated by suction filtration and dried under vacuum overnight at room temperature**:** $M_w$ = 291,000, MWD = 1.39 (SEC-MALLS), HEMA/MMA = 46/54 (mol%). $^1$H NMR (500.16 MHz, $CDCl_3/CD_3OD$ = 1/1, $Si(CH_3)_4$ = 0 ppm): δ (ppm) = 4.33-3.91 ($-CH_2-CH_2-OH$), 3.88-3.72 ($-CH_2-CH_2-OH$), 3.72-3.55 ($-OCH_3$), 2.31-1.40 ($-CH_2-$), 1.39-0.80 ($-CH_3$).

**star-H71M29**: A procedure similar to that described for star-H47M53 was applied to PTMSOEMA (8.13 g, 0.502 mmol, $M_n$ = 16,200, $M_w/M_n$ = 1.30), PMMA (1.39 g, 0.167 mmol, $M_n$ = 8,300, $M_w/M_n$ = 1.25), $Ru(Ind)Cl(PPh_3)_2$ (0.156 g, 0.134 mmol), toluene (68.8 mL), $n$-$Bu_3N$ (0.32 mL, 1.34 mmol), EGDMA (1.26 mL, 6.68 mmol). The reaction time for the star polymer formation was 52h. $M_w$ = 227,000, MWD = 1.17 (SEC-MALLS), PHEMA/PMMA = 71/29 (mol%). $^1$H NMR (500.16 MHz, $CDCl_3/CD_3OD$ = 1/1, $Si(CH_3)_4$ = 0 ppm): δ (ppm) = 4.27-3.90 ($-CH_2-CH_2-OH$), 3.88-3.72 ($-CH_2-CH_2-OH$), 3.72-3.53 ($-OCH_3$), 2.31-1.40 ($-CH_2-$), 1.40-0.80 ($-CH_3$).

**star-H22M78**: A procedure similar to that described for star-H47M53 was applied to PTMSOEMA (2.71 g, 0.167 mmol, $M_n$ = 16,200, $M_w/M_n$ = 1.30), PMMA (4.16 g, 0.502 mmol, $M_n$ = 8,300, $M_w/M_n$ = 1.25), $Ru(Ind)Cl(PPh_3)_2$ (0.156 g, 0.134 mmol), toluene (71.4 mL), $n$-$Bu_3N$ (0.32 mL, 1.34 mmol), EGDMA (1.26 mL, 6.68 mmol). The reaction time for the star polymer formation was 52 h. $M_w$ =250,000, MWD =1.23 (SEC-MALLS), HEMA/MMA = 22/78 (mol%). $^1$H NMR (500.16 MHz, $CDCl_3/CD_3OD$ = 1/1, $Si(CH_3)_4$ = 0 ppm): δ (ppm) = 4.33-3.95 ($-CH_2-$

$CH_2$-OH), 3.91-3.74 (-$CH_2$-C$H_2$-OH), 3.74-3.54 (-OC$H_3$), 2.24-1.38 (-C$H_2$-), 1.38-0.80 (-C$H_3$).

### *1-3. Synthesis of linear PHEMA*

**lin-PHEMA 27k:** A lin-PHEMA 27k was prepared by the deprotection of PTMSOEMA (precursor PTMSOEMA star). The TMS groups of the polymer were removed by HCl to give PHEMA from PTMSOEMA ($M_w$ = 26,700): $M_n$ = 20,000, MWD =1.34 (SEC). $^1$H NMR (500.16 MHz, $CD_3OD$, Si(C$H_3$)$_4$ = 0 ppm): δ (ppm) = 4.18-3.91 (-C$H_2$-$CH_2$-OH), 3.91-3.67 (-$CH_2$-C$H_2$-OH), 2.20-1.44 (-C$H_2$-), 1.44-0.71 (-C$H_3$).

**lin-PHEMA 290k:** PHEMA with higher MW (lin-PHEMA 290k) was prepared by free radical polymerization. AIBN (100 mg, 0.61 mmol), ethanol (22.6 mL), and HEMA (7.40 mL, 61 mmol) were added into a 100mL flask equipped with a three-way stopcock. After purged by argon bubbling for 15 min, the solution mixture was placed in an oil bath at 65 ºC for 2 h. The polymer was precipitated into acetone and collected by centrifugation. The precipitate was dried overnight under vacuum at room temperature: $M_n$ = 162,000, $M_w$ = 286,000, MWD = 1.77 (SEC). $^1$H NMR (500.16 MHz, $CD_3OD$, Si(C$H_3$)$_4$ = 0 ppm): δ (ppm) = 4.18-3.91 (-C$H_2$-$CH_2$-OH), 3.91-3.67 (-$CH_2$-C$H_2$-OH), 2.20-1.44 (-C$H_2$-), 1.44-0.71 (-C$H_3$).

### *1-4. Synthesis of PHEMA/PMMA diblock copolymer (lin-Block)*

PHEMA/PMMA diblock copolymer was synthesized under argon by the similar procedure described for synthesis of precursor PMMA. Ru(Ind)Cl($PPh_3$)$_2$ (15.5 mg, 0.0180 mmol), toluene (3.41 mL), *n*-$Bu_3N$ (0.159 mL, 0.180 mmol), TMSOEMA (3.92 mL, 18.0 mmol), and PMMA macroinitiator ($M_n$ = 8,300, $M_w$ = 10,400, MWD = 1.25, 1.49 g, 0.18 mmol) were added in a baked glass tube. The mixtures were placed in an oil bath at 80 ºC for 19 h. The diblock copolymer was precipitated into hexane, The Ru-complex was removed by silica-gel and alumina column chromatography eluted with toluene, and the TMS groups of polymer were removed by HCl. $M_n$ = 26,300, $M_w$ = 32,500, MWD = 1.23 (SEC). HEMA/MMA = 52/48 (mol%). $^1$H NMR (500.16 MHz, $CD_3OD/CDCl_3$ = 1/1, $Si(CH_3)_4$ = 0 ppm): δ (ppm) = 4.21-3.94 ($-CH_2-CH_2-OH$), 3.80-3.40 ($-CH_2-CH_2-OH$), 3.50-3.40 ($-OCH_3$), 2.16-1.36 ($-CH_2-$), 1.36-0.70 ($-CH_3$).

***1-5. PHEMA/PMMA random copolymer (lin-Random)***

PHEMA/PMMA random copolymer was prepared by the same procedure described for polymerization of precursor PMMA. The monomers MMA and TMSOEMA were mixed in the polymerization to give a random copolymer. $M_n$ = 24,500, $M_w$ = 29,200, MWD = 1.19 (SEC), HEMA/MMA = 51/49 (mol%). $^1$H NMR (500.16 MHz, $CD_3OD/CDCl_3$ = 1/1, $Si(CH_3)_4$ = 0 ppm): δ (ppm) = 4.21-3.94 ($-CH_2-CH_2-OH$), 3.80-3.40 ($-CH_2-CH_2-OH$), 3.50-3.40 ($-O-CH_3$), 2.16-1.36 ($-CH_2-$), 1.36-0.70 ($-CH_3$).

## 2. Calculation of number of arms

### 2-1. Determination of the ratio of arms in a star polymer

The HEMA and MMA monomer ratio of star polymers was determined by comparing the integrated intensities of the $^1$H NMR signals from the side chain's ethylene group of HEMA (4.28-3.74 ppm) relative to the methyl of MMA (3.74-3.56 ppm). The PHEMA and PMMA ratio of star polymers was determined by the following relationship:

$$\frac{\text{HEMA ratio}}{\text{DP}_\text{n}\text{ of PHEMA}} : \frac{\text{MMA ratio}}{\text{DP}_\text{n}\text{ of PMMA}} = \text{PHEMA} : \text{PMMA}$$

**2-2. Determination of the number of arms in star polymers**

The numbers of arms in star polymers were determined based on $^1$H NMR, SEC and SEC-MALLS analyses of precursor polymers and star polymers.

(a) star-PHEMA

The number of PHEMA arms in star-PHEMA, *f*(star-PHEMA), was estimated by molecular weights of star-PTMSOEMA and lin-PTMSOEMA. We assumed that cross-linkers quantitatively reacted with precursor polymers to give star-PTMSOEMA, and the protecting groups (TMS) were also removed quantitatively.

$$f(\text{star-PHEMA}) = f(\text{star-PTMSOEMA})$$

$$= \frac{[M_\text{w}(\text{star-PTMSOEMA}) \times \text{WF(PTMSOEMA)}]}{M_\text{w}(\text{lin-PTMSOEMA})}$$

where *f*(star-PTMSOEMA), $M_\text{w}$(star-PTMSOEMA), WF(PTMSOEMA), $M_\text{w}$(lin-PTMSOEMA) represent the number of arms in star-PTMSOEMA, weight averaged molecular weight of star-PTMSOEMA measured by SEC-MALLS, weight fraction of PTMSOEMA arms in star-PTMSOEMA, and weight averaged molecular weight of lin-PTMSOEMA ($M_\text{w}$ = 21,400) measured by SEC, respectively. The weight fraction of PTMSOEMA was calculated as follows:

$$\text{MF(PTMSOEMA)} = \frac{\text{W(PTMSOEMA)} \times \text{yield of star-PTMSOEMA}}{[\text{W(PTMSOEMA)} \times \text{yield of star-PTMSOEMA} + \text{W(EGDMA)}]}$$

where W(PTMSOEMA) and W(EGDMA) mean the weights of PTMSOEMA and EGDMA in the reaction solution. All the information was summarized in Table S1.

(b) star-PMMA

The number of PMMA arms in star-PMMA, $f$(star-PMMA), was estimated by molecular weights of star-PMMA and lin-PMMA. We assumed that cross-linkers quantitatively reacted with precursor polymers to give star-PMMA.

$$f(\text{star-PMMA}) = \frac{M_{\mathrm{w}}(\text{star-PMMA}) \times \mathrm{WF(PMMA)}}{M_{\mathrm{w}}(\text{lin-PMMA 10k})}$$

where $f$(star-PMMA), $M_{\mathrm{w}}$(star-PMMA), WF(PMMA), $M_{\mathrm{w}}$(lin-PMMA) represent the number of arms in star-PMMA, weight averaged molecular weight of star-PMMA measured by SEC-MALLS, weight fraction of PMMA arms in star-PMMA, and weight averaged molecular weight of lin-PMMA ($M_{\mathrm{w}}$ = 10,400) measured by SEC, respectively. The weight fraction of PMMA was calculated as follows:

$$\mathrm{WF(PMMA)} = \frac{\mathrm{W(PMMA)} \times \text{yield of star-PMMA}}{[\mathrm{W(PMMA)} \times \text{yield of star-PMMA} + \mathrm{W(EGDMA)}]}$$

where W(PMMA) and W(EGDMA) mean the weights of PMMA and EGDMA in the reaction solution. All the information was summarized in Table S1.

(c) Heteroarm star polymers

The number of arms in heteroarm star polymers, $f(\mathrm{H\underline{X}M\underline{Y}})$, should be determined by the flowing equation:

$$f(\text{star-HXMY}) = f(\text{star-PHEMA}) + f(\text{star-PMMA})$$

$$= \frac{M_\mathrm{w}(\text{star-HXMY}) \times \mathrm{WF}(\text{PHEMA})}{M_\mathrm{w}(\text{lin-PHEMA 27k})} + \frac{M_\mathrm{w}(\text{star-HXMY}) \times \mathrm{WF}(\text{PMMA})}{M_\mathrm{w}(\text{lin-PMMA 10k})}$$

where $f$(PHEMA), $f$(PMMA), WF(PHEMA), WF(PMMA), $M_\mathrm{w}$(lin-PHEMA 27k), and $M_\mathrm{w}$(lin-PMMA 10k) represent the number of arms of PHEMA and PMMA in the heteroarm star polymer, weight fraction of PHEMA and PMMA, weight averaged molecular weight of PHEMA and PMMA, respectively. ($M_\mathrm{w}$(PHEMA) = 26,700, $M_\mathrm{w}$(PMMA) = 10,400). However, it is difficult to determine of $M_\mathrm{w}$(PHEMA) by means of SEC because the exclusion volume of PHEMA chain was much larger than that of PMMA chain having the same molecular weight. We thus estimated $f$(PHEMA) by the following equation:

$$f(\text{PHEMA}) = f(\text{PMMA}) \times \frac{r(\text{PHEMA})}{r(\text{PMMA})}$$

where $r$(PHEMA) and $r$(PMMA) represent the ratio of PHEMA and PMMA arms in the heteroarm star polymer *(NOT the ratio of monomers)*. They can be calculated the ratio of each monomer in $^1$H NMR spectra and degree of polymerization of each polymer. $f(\mathrm{H\underline{X}M\underline{Y}})$ can be thus determined by the following equation:

$$f(\text{star-HXMY}) = f(\text{star-PHEMA}) \times \frac{r(\text{PHEMA})}{r(\text{PMMA})} + f(\text{PMMA})$$

All the information was summarized in Table S2.

Table S1. The number of PHEMA and PMMA arms in homo-star polymers.

| Star polymers | $M_\mathrm{w}$ (star-polymer) | WF (PTMS-OEMA) | WF (PMMA) | W (PTMS-OEMA) (g) | W (PMMA) (g) | W (EGDMA) (g) | Yield of star (%) | Monomer mole ratio (HEMA:MMA) | Polymer mole ratio (PHEMA:PMMA) | Total number of arms | PHEMA arms | PMMA arms |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| star-PTMS-OEMA | 484,000 | 0.87 | - | 10.8 | - | 1.33 | 88 | 1.00:0 | 1.00:0 | 20 | 20 | 0 |
| star-PMMA | 209,000 | - | 0.80 | - | 14.9 | 3.57 | 98 | 0:1.00 | 0:1.00 | 16 | 0 | 16 |

Table S2. The number of PHEMA and PMMA arms in heteroarm star polymers.

| Star polymers | $M_w$ (star-H$\underline{X}$M$\underline{Y}$)[a] | WF (PMMA) | $r$ (PHEMA) | $r$ (PMMA) | Yield of star (%) | Monomer mole ratio (HEMA:MMA) [a] | Polymer mole ratio (PHEMA:PMMA) | Total number of arms, $f$(H$\underline{X}$M$\underline{Y}$) | PHEMA arms, $f$(PHEMA) | PMMA arms, $f$(PMMA) |
|---|---|---|---|---|---|---|---|---|---|---|
| star-H71M29 | 227,000 | 0.17 | 0.71 | 0.29 | 95 | 0.70:0.30 | 0.71:0.29 | 14 | 10 | 4 |
| star-H47M53 | 291,000 | 0.36 | 0.47 | 0.53 | 93 | 0.46:0.54 | 0.47:0.53 | 19 | 9 | 10 |
| star-H22M78 | 250,000 | 0.57 | 0.22 | 0.78 | 96 | 0.22:0.78 | 0.22:0.78 | 18 | 4 | 14 |

a) determined by $^1$H NMR analysis of depotected star-polymers.

## 3. AFM and SEM images of coated surfaces

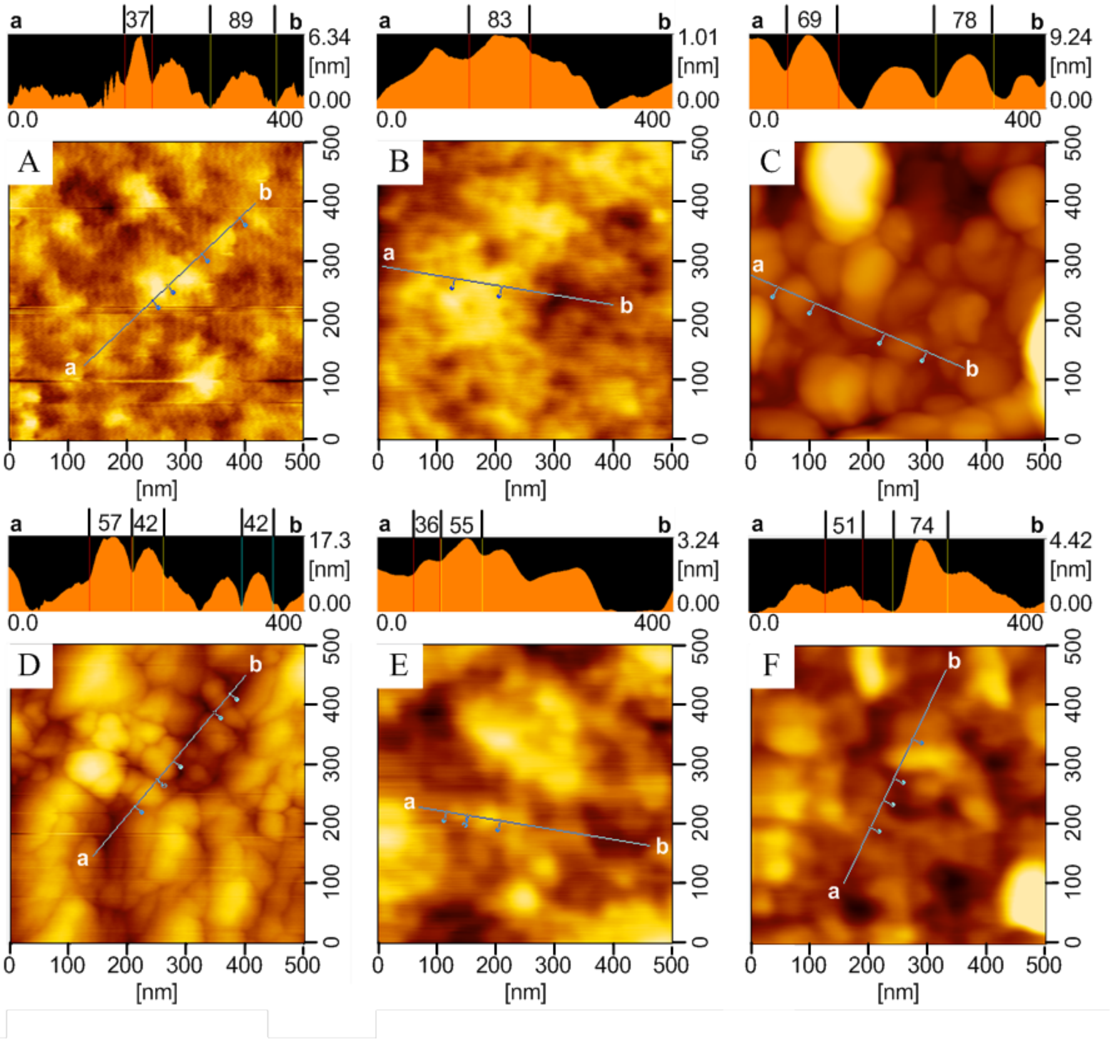

**Fig. S1.** Representative AFM images of each polymer film prepared under dry conditions. Substrate = PET sheet. (A) PET surface, (B) lin-PHEMA 27k, (C) star-PHEMA, (D) star-H47M53, (E) star-PMMA, (F) lin-Block.

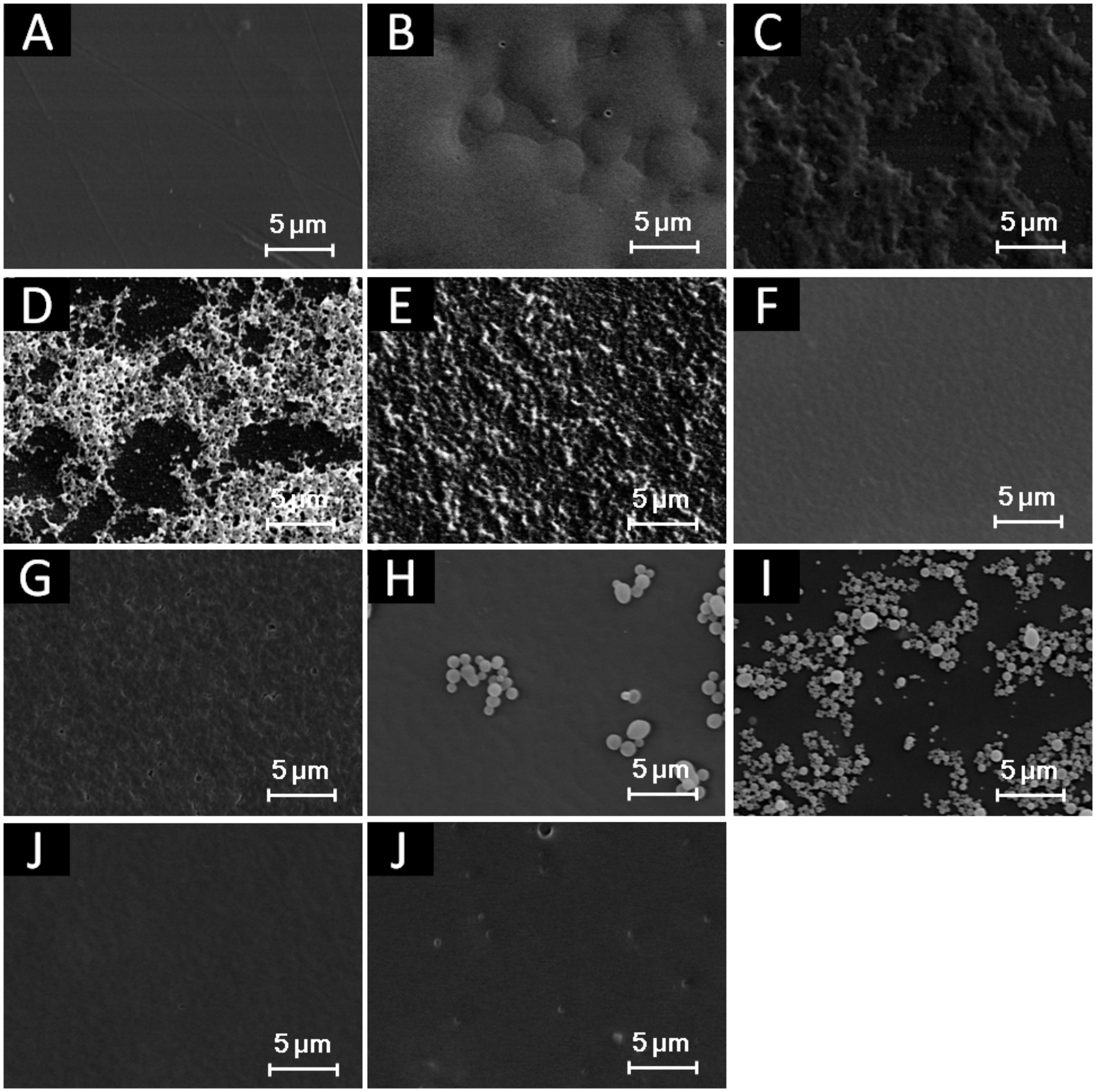

**Fig. S2.** Representative SEM images of each polymer film prepared under dry conditions. Substrate = PET sheet. (A) PET surface, (B) lin-PHEMA 27k, (C) lin-PHEMA 290k, (D) star-PHEMA, (E) star-H71M29, (F) star-H47M53, (G) star-H22M78, (H) lin-PMMA 10k, (I) star-PMMA, (J) lin-Block, (K) lin-Block.

## **4.** SEM images of adherent platelets

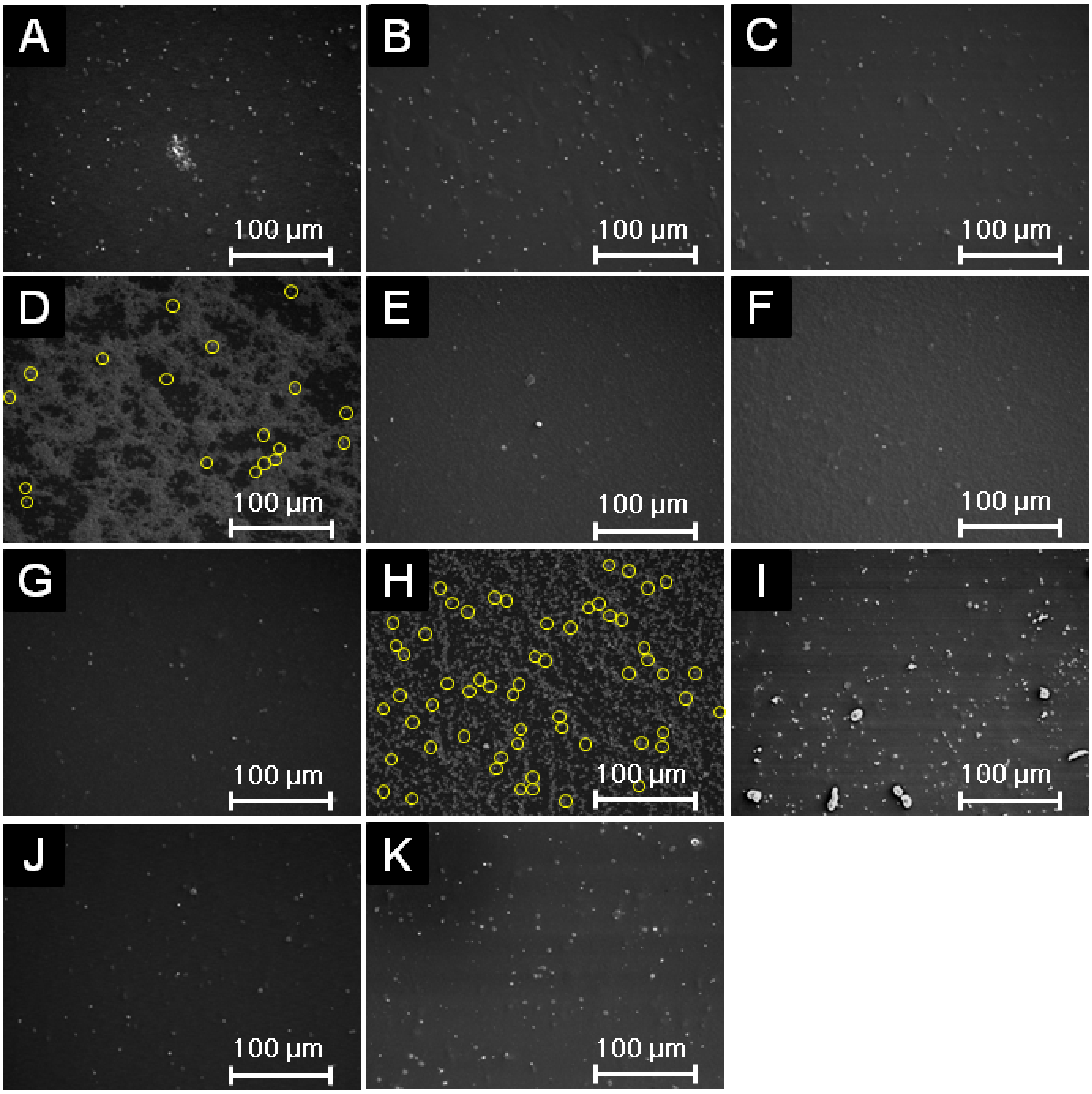

**Fig. S3.** Adherent platelet images on each polymer surface: Substrate = PET sheet; Incubation in PRP at 37 ºC for 30min; (A) PET surface, (B) lin-PHEMA 27k, (C) lin-PHEMA 290k, (D) star-PHEMA, (E) star-H71M29, (F) star-H47M53, (G) star-H22M78, (H) star-PMMA, (I) lin-PMMA 10k, (J) lin-Block, (K) lin-Random.

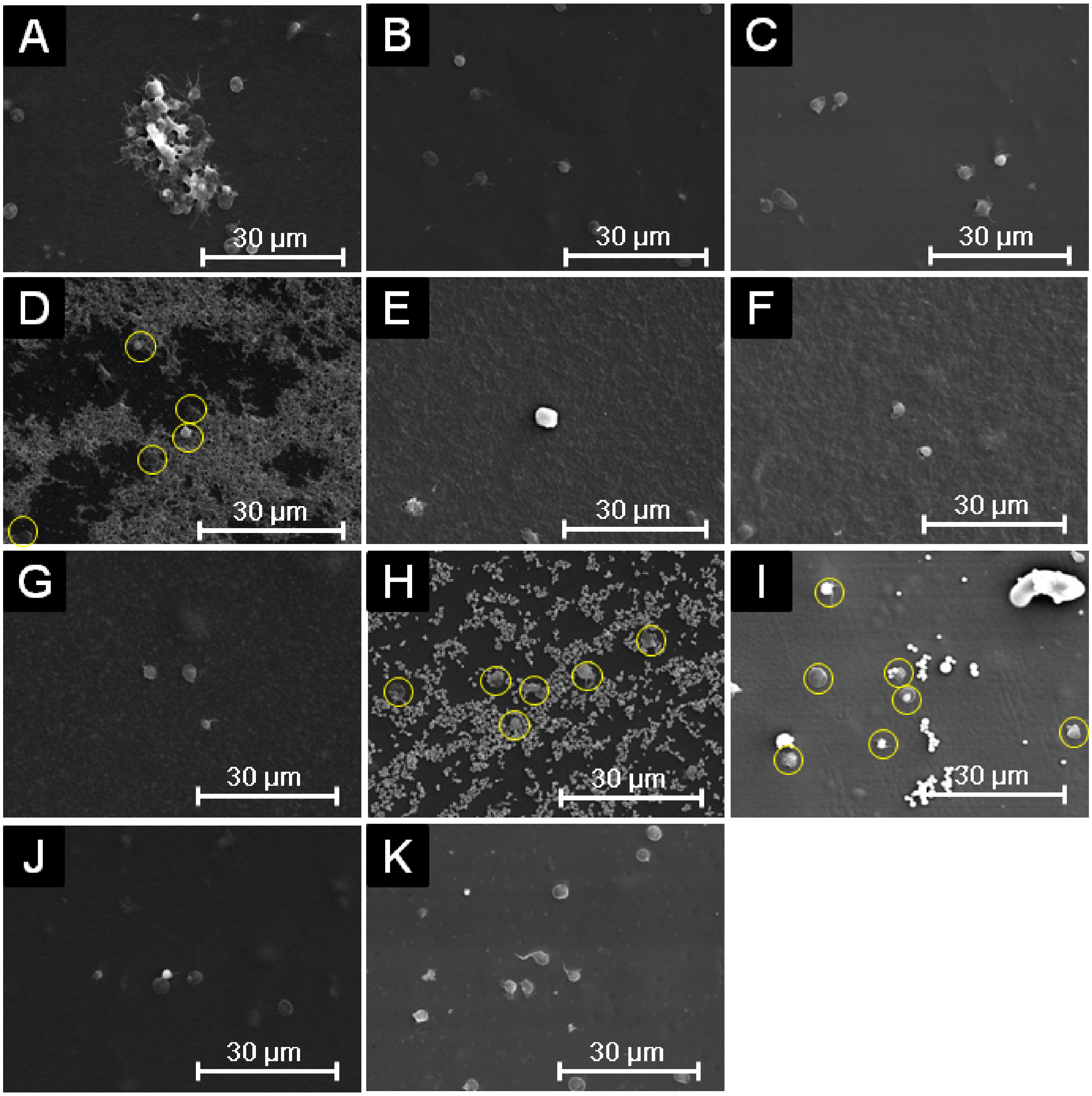

**Fig. S4.** Adherent platelet images magnified on each polymer surface: Substrate = PET sheet. Incubation in PRP at 37 ºC for 30min. (A) PET surface, (B) lin-PHEMA 27k, (C) lin-PHEMA 290k, (D) star-PHEMA, (E) star-H71M29, (F) star-H47M53, (G) star-H22M78, (H) star-PMMA, (I) lin-PMMA 10k, (J) lin-Block, (K) lin-Random.

## 5. SEM images of adherent bacteria

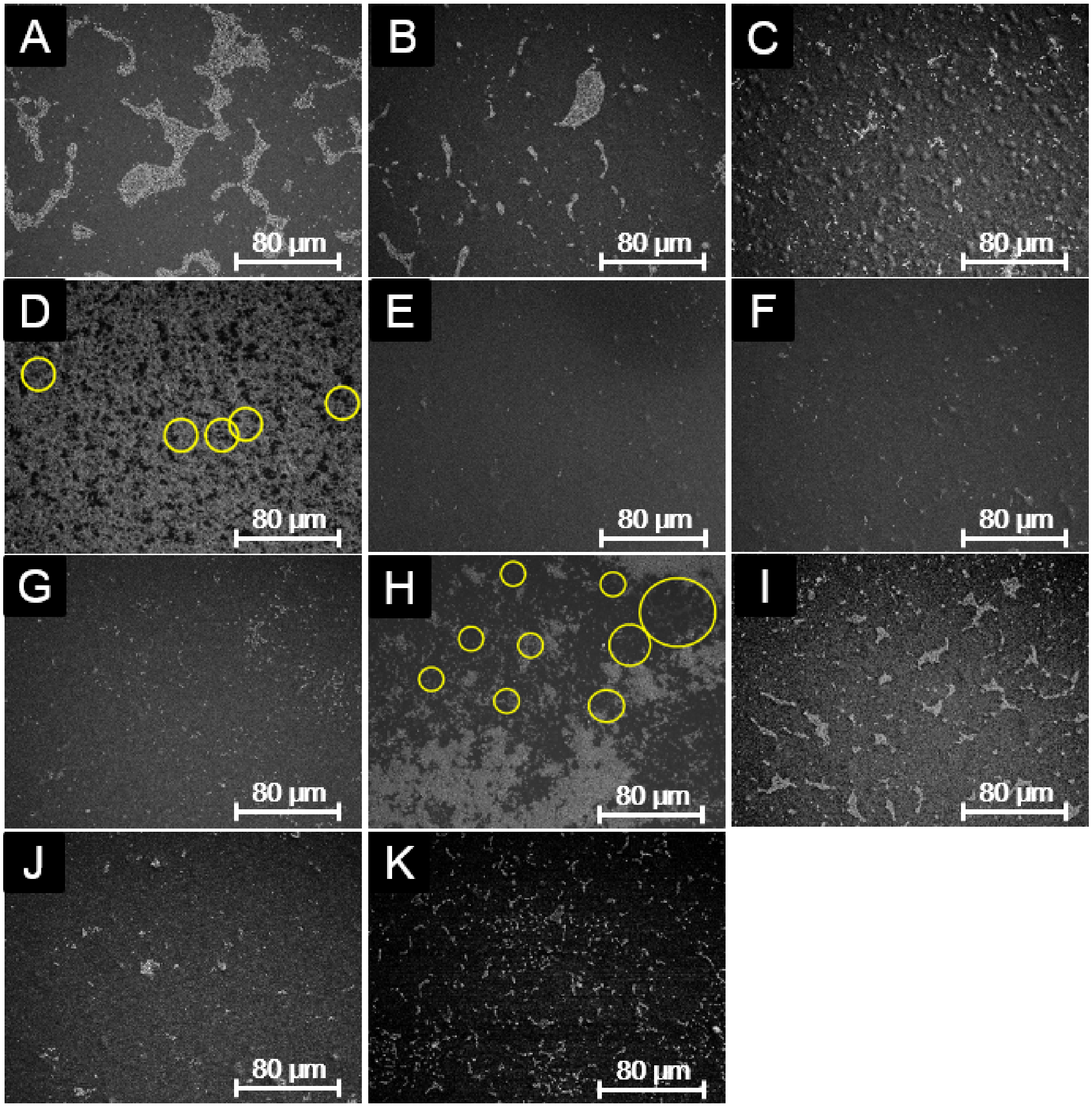

**Fig. S5.** Adherent bacterial images on each polymer surface. Substrate = PET sheet; Incubation = *E. coli* in MH broth at 37 ºC for 20 h. (A) PET surface, (B) lin-PHEMA 290k, (C) lin-PHEMA 14k,(D) star-PHEMA, (E) star-H71M29, (F) star-H47M53, (G) star-H22M78, (H) star-PMMA, (I) lin-PMMA 10k, (J) lin-Block, (K) lin-Random.

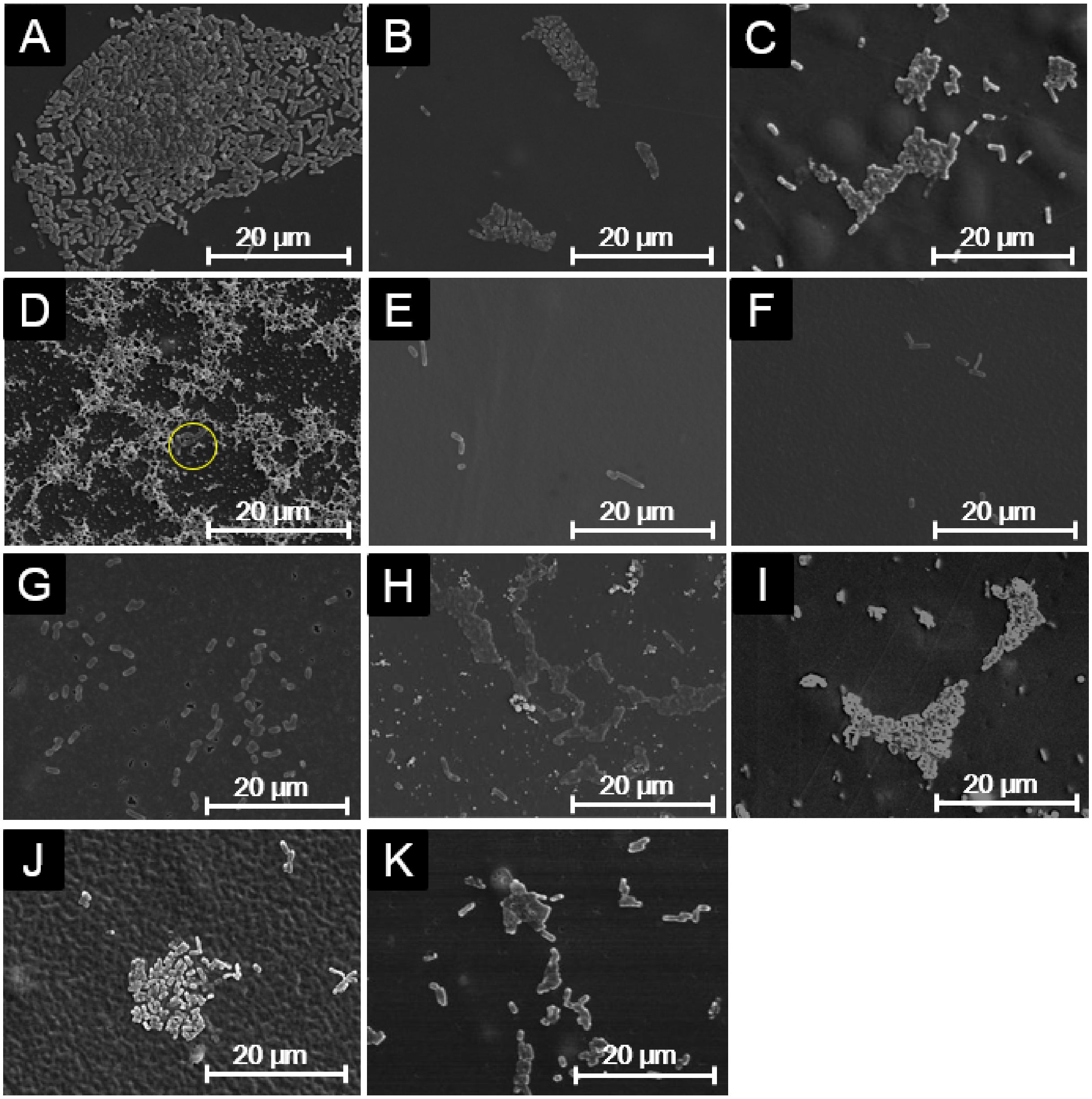

**Fig. S6.** Adherent bacterial images magnified on each polymer surface Substrate = PET sheet; Incubation = *E. coli* in MH broth at 37 ºC for 20 h. (A) PET surface, (B) lin-PHEMA 27k, (C) lin-PHEMA 290k, (D) star-PHEMA, (E) star-H71M29, (F) star-H47M53, (G) star-H22M78, (H) star-PMMA, (I) lin-PMMA 10k, (J) lin-Block, (K) lin-Random.