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# Polymer Microarray with Novel Substrate for Cellular Adhesion Study

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**Electronic Supplementary Information**

| PU- | Polyol | Polyol MW (Da) | Dis. | Ext. | x (Polyol) | x (Dis) | x (Ext) | Average Number of Epithelial cells | Standard Deviation |
|---|---|---|---|---|---|---|---|---|---|
| 3 | PEG | 400 | HDI | none | 0.485 | 0.515 | 0.000 | 0.3 | 0.5 |
| 4 | PPG | 2000 | HDI | none | 0.485 | 0.515 | 0.000 | 1.8 | 1.7 |
| 8 | PEG | 400 | BICH | none | 0.485 | 0.515 | 0.000 | 0.0 | 0.0 |
| 9 | PPG | 2000 | BICH | none | 0.485 | 0.515 | 0.000 | 11.5 | 2.9 |
| 10 | PTMG | 2000 | BICH | none | 0.485 | 0.515 | 0.000 | 3.5 | 2.6 |
| 12 | PEG | 900 | TDI | none | 0.485 | 0.515 | 0.000 | 19.3 | 8.3 |
| 13 | PEG | 400 | TDI | none | 0.485 | 0.515 | 0.000 | 37.0 | 23.3 |
| 14 | PPG | 2000 | TDI | none | 0.485 | 0.515 | 0.000 | 23.3 | 18.5 |
| 15 | PTMG | 2000 | TDI | none | 0.485 | 0.515 | 0.000 | 29.3 | 14.2 |
| 16 | PEG | 2000 | MDI | none | 0.485 | 0.515 | 0.000 | 0.0 | 0.0 |
| 17 | PEG | 900 | MDI | none | 0.485 | 0.515 | 0.000 | 16.8 | 10.8 |
| 18 | PEG | 400 | MDI | none | 0.485 | 0.515 | 0.000 | 143.5 | 41.3 |
| 19 | PPG | 2000 | MDI | none | 0.485 | 0.515 | 0.000 | 63.5 | 20.0 |
| 20 | PTMG | 2000 | MDI | none | 0.485 | 0.515 | 0.000 | 64.8 | 10.9 |
| 22 | PEG | 900 | PDI | none | 0.485 | 0.515 | 0.000 | 2.3 | 2.9 |
| 23 | PEG | 400 | PDI | none | 0.485 | 0.515 | 0.000 | 87.3 | 19.7 |
| 24 | PPG | 2000 | PDI | none | 0.485 | 0.515 | 0.000 | 30.0 | 14.3 |
| 25 | PTMG | 2000 | PDI | none | 0.485 | 0.515 | 0.000 | 17.8 | 14.7 |
| 28 | PEG | 400 | HMDI | none | 0.485 | 0.515 | 0.000 | 2.8 | 3.6 |
| 29 | PPG | 2000 | HMDI | none | 0.485 | 0.515 | 0.000 | 1.0 | 0.8 |
| 30 | PTMG | 2000 | HMDI | none | 0.485 | 0.515 | 0.000 | 3.5 | 4.4 |
| 31 | PEG | 2000 | HDI | BD | 0.250 | 0.520 | 0.230 | 0.0 | 0.0 |
| 33 | PEG | 900 | HDI | BD | 0.250 | 0.520 | 0.230 | 0.3 | 0.5 |
| 35 | PEG | 400 | HDI | BD | 0.250 | 0.520 | 0.230 | 8.5 | 3.7 |
| 37 | PPG | 2000 | HDI | BD | 0.250 | 0.520 | 0.230 | 23.0 | 6.8 |
| 38 | PPG | 2000 | HDI | ED | 0.250 | 0.520 | 0.230 | 0.0 | 0.0 |
| 40 | PTMG | 2000 | HDI | ED | 0.250 | 0.520 | 0.230 | 0.0 | 0.0 |
| 41 | PEG | 2000 | BICH | BD | 0.250 | 0.520 | 0.230 | 0.0 | 0.0 |
| 43 | PEG | 900 | BICH | BD | 0.250 | 0.520 | 0.230 | 0.0 | 0.0 |
| 45 | PEG | 400 | BICH | BD | 0.250 | 0.520 | 0.230 | 0.8 | 1.5 |
| 46 | PEG | 400 | BICH | ED | 0.250 | 0.520 | 0.230 | 12.5 | 3.7 |
| 47 | PPG | 2000 | BICH | BD | 0.250 | 0.520 | 0.230 | 16.5 | 12.6 |
| 48 | PPG | 2000 | BICH | ED | 0.250 | 0.520 | 0.230 | 6.5 | 6.2 |
| 49 | PTMG | 2000 | BICH | BD | 0.250 | 0.520 | 0.230 | 41.3 | 30.5 |
| 50 | PTMG | 2000 | BICH | ED | 0.250 | 0.520 | 0.230 | 48.0 | 55.6 |
| 53 | PEG | 900 | TDI | BD | 0.250 | 0.520 | 0.230 | 0.3 | 0.5 |
| 55 | PEG | 400 | TDI | BD | 0.250 | 0.520 | 0.230 | 54.5 | 21.0 |
| 57 | PPG | 2000 | TDI | BD | 0.250 | 0.520 | 0.230 | 101.8 | 32.1 |
| 59 | PTMG | 2000 | TDI | BD | 0.250 | 0.520 | 0.230 | 121.8 | 45.3 |
| 61 | PEG | 2000 | MDI | BD | 0.250 | 0.520 | 0.230 | 5.0 | 3.2 |
| 63 | PEG | 900 | MDI | BD | 0.250 | 0.520 | 0.230 | 23.0 | 12.6 |
| 65 | PEG | 400 | MDI | BD | 0.250 | 0.520 | 0.230 | 79.0 | 23.2 |
| 67 | PPG | 2000 | MDI | BD | 0.250 | 0.520 | 0.230 | 83.5 | 22.9 |
| 69 | PTMG | 2000 | MDI | BD | 0.250 | 0.520 | 0.230 | 61.5 | 20.0 |
| 71 | PEG | 2000 | PDI | BD | 0.250 | 0.520 | 0.230 | 0.0 | 0.0 |
| 73 | PEG | 900 | PDI | BD | 0.250 | 0.520 | 0.230 | 0.0 | 0.0 |
| 77 | PPG | 2000 | PDI | BD | 0.250 | 0.520 | 0.230 | 64.8 | 74.8 |
| 79 | PTMG | 2000 | PDI | BD | 0.250 | 0.520 | 0.230 | 30.5 | 15.2 |
| 81 | PEG | 2000 | HMDI | BD | 0.250 | 0.520 | 0.230 | 0.0 | 0.0 |
| 83 | PEG | 900 | HMDI | BD | 0.250 | 0.520 | 0.230 | 1.0 | 2.0 |
| 85 | PEG | 400 | HMDI | BD | 0.250 | 0.520 | 0.230 | 103.8 | 29.4 |
| 87 | PPG | 2000 | HMDI | BD | 0.250 | 0.520 | 0.230 | 11.8 | 20.2 |
| 89 | PTMG | 2000 | HMDI | BD | 0.250 | 0.520 | 0.230 | 24.8 | 20.6 |
| 91 | PTMG | 650 | HDI | BD | 0.485 | 0.515 | 0.000 | 2.0 | 3.4 |
| 92 | PTMG | 1000 | HDI | BD | 0.485 | 0.515 | 0.000 | 41.0 | 35.4 |
| 93 | PTMG | 650 | BICH | BD | 0.485 | 0.515 | 0.000 | 5.0 | 4.2 |
| 94 | PTMG | 1000 | BICH | BD | 0.485 | 0.515 | 0.000 | 24.8 | 17.9 |
| 95 | PTMG | 650 | MDI | BD | 0.485 | 0.515 | 0.000 | 45.5 | 20.4 |
| 96 | PTMG | 1000 | MDI | BD | 0.485 | 0.515 | 0.000 | 83.3 | 18.2 |

| PU- | Polyol | Polyol MW (Da) | Dis. | Ext. | x (Polyol) | x (Dis) | x (Ext) | Average Nbre of Epithelial cells | Standard Deviation |
|---|---|---|---|---|---|---|---|---|---|
| 97 | PHNGAD | 1800 | BICH | DMAPD | 0.250 | 0.520 | 0.230 | 18.5 | 12.1 |
| 123 | PPG | 2000 | MDI | DMAPD | 0.250 | 0.520 | 0.230 | 2.0 | 1.2 |
| 158 | PTMG | 250 | MDI | OFHD | 0.250 | 0.520 | 0.230 | 7.8 | 9.7 |
| 159 | PTMG | 250 | MDI | BD | 0.250 | 0.520 | 0.230 | 108.5 | 27.0 |
| 160 | PTMG | 250 | MDI | EG | 0.250 | 0.520 | 0.230 | 132.5 | 18.0 |
| 161 | PTMG | 650 | MDI | EG | 0.250 | 0.520 | 0.230 | 150.3 | 21.8 |
| 162 | PTMG | 1000 | MDI | EG | 0.250 | 0.520 | 0.230 | 83.3 | 31.8 |
| 163 | PTMG | 2000 | MDI | EG | 0.250 | 0.520 | 0.230 | 106.3 | 44.9 |
| 164 | PTMG | 250 | MDI | PG | 0.250 | 0.520 | 0.230 | 119.5 | 35.4 |
| 165 | PTMG | 650 | MDI | PG | 0.250 | 0.520 | 0.230 | 144.3 | 4.3 |
| 166 | PTMG | 1000 | MDI | PG | 0.250 | 0.520 | 0.230 | 85.0 | 32.5 |
| 167 | PTMG | 2000 | MDI | PG | 0.250 | 0.520 | 0.230 | 74.3 | 22.1 |
| 168 | PTMG | 250 | BICH | none | 0.485 | 0.515 | 0.000 | 59.3 | 50.3 |
| 169 | PTMG | 650 | BICH | none | 0.485 | 0.515 | 0.000 | 0.8 | 1.0 |
| 170 | PTMG | 1000 | BICH | none | 0.485 | 0.515 | 0.000 | 0.0 | 0.0 |
| 171 | PTMG | 250 | HDI | none | 0.485 | 0.515 | 0.000 | 81.8 | 24.8 |
| 172 | PTMG | 650 | HDI | none | 0.485 | 0.515 | 0.000 | 77.8 | 48.4 |
| 174 | PTMG | 250 | MDI | none | 0.485 | 0.515 | 0.000 | 59.8 | 55.7 |
| 175 | PTMG | 650 | MDI | none | 0.485 | 0.515 | 0.000 | 106.0 | 17.8 |
| 176 | PTMG | 1000 | MDI | none | 0.485 | 0.515 | 0.000 | 94.0 | 15.8 |
| 177 | PTMG | 250 | HDI | NMPD | 0.250 | 0.520 | 0.230 | 113.0 | 8.8 |
| 178 | PTMG | 1000 | HDI | NMPD | 0.250 | 0.520 | 0.230 | 109.8 | 14.3 |
| 179 | PTMG | 2000 | HDI | NMPD | 0.250 | 0.520 | 0.230 | 22.3 | 16.9 |
| 180 | PTMG | 1000 | BICH | NMPD | 0.250 | 0.520 | 0.230 | 31.0 | 24.8 |
| 181 | PTMG | 2000 | BICH | NMPD | 0.250 | 0.520 | 0.230 | 30.0 | 19.7 |
| 182 | PTMG | 650 | MDI | NMPD | 0.250 | 0.520 | 0.230 | 142.3 | 18.3 |
| 183 | PTMG | 1000 | MDI | NMPD | 0.250 | 0.520 | 0.230 | 95.3 | 29.7 |
| 184 | PTMG | 2000 | MDI | NMPD | 0.250 | 0.520 | 0.230 | 132.3 | 9.9 |
| 185 | PHNAD | 900 | MDI | OFHD | 0.170 | 0.520 | 0.330 | 3.0 | 1.6 |
| 186 | PTMG | 650 | BICH | OFHD | 0.250 | 0.520 | 0.230 | 29.8 | 32.7 |
| 187 | PTMG | 1000 | BICH | OFHD | 0.250 | 0.520 | 0.230 | 80.3 | 58.4 |
| 188 | PTMG | 2000 | BICH | OFHD | 0.250 | 0.520 | 0.230 | 5.5 | 3.1 |
| 189 | PPG | 1000 | BICH | OFHD | 0.170 | 0.520 | 0.330 | 7.5 | 8.7 |
| 190 | PTMG | 650 | HDI | OFHD | 0.250 | 0.520 | 0.230 | 59.0 | 59.3 |
| 191 | PTMG | 1000 | HDI | OFHD | 0.250 | 0.520 | 0.230 | 106.5 | 16.5 |
| 192 | PTMG | 2000 | HDI | OFHD | 0.250 | 0.520 | 0.230 | 80.8 | 26.5 |
| 193 | PPG | 1000 | MDI | DMAPD | 0.170 | 0.520 | 0.330 | 133.5 | 22.0 |
| 194 | PTMG | 650 | MDI | OFHD | 0.250 | 0.520 | 0.230 | 55.8 | 19.7 |
| 195 | PTMG | 1000 | MDI | OFHD | 0.250 | 0.520 | 0.230 | 141.0 | 40.1 |
| 196 | PTMG | 2000 | MDI | OFHD | 0.250 | 0.520 | 0.230 | 117.5 | 23.6 |
| 197 | PTMG | 650 | BICH | DHM | 0.250 | 0.520 | 0.230 | 9.3 | 11.5 |
| 198 | PTMG | 1000 | BICH | DHM | 0.250 | 0.520 | 0.230 | 19.8 | 18.1 |
| 199 | PTMG | 2000 | BICH | DHM | 0.250 | 0.520 | 0.230 | 70.5 | 9.7 |
| 200 | PTMG | 650 | HDI | DHM | 0.250 | 0.520 | 0.230 | 135.8 | 1.3 |
| 201 | PTMG | 1000 | HDI | DHM | 0.250 | 0.520 | 0.230 | 104.0 | 17.6 |
| 202 | PTMG | 2000 | HDI | DHM | 0.250 | 0.520 | 0.230 | 20.3 | 26.9 |
| 203 | PTMG | 650 | MDI | DHM | 0.250 | 0.520 | 0.230 | 46.3 | 32.0 |
| 204 | PTMG | 1000 | MDI | DHM | 0.250 | 0.520 | 0.230 | 88.3 | 14.2 |
| 205 | PTMG | 2000 | MDI | DHM | 0.250 | 0.520 | 0.230 | 69.3 | 2.6 |
| 206 | PPG | 1000 | HDI | OFHD | 0.250 | 0.520 | 0.230 | 25.3 | 33.2 |
| 207 | PPG | 1000 | BICH | OFHD | 0.250 | 0.520 | 0.230 | 3.8 | 7.5 |
| 208 | PPG | 1000 | MDI | OFHD | 0.250 | 0.520 | 0.230 | 42.0 | 35.2 |
| 209 | PPG | 1000 | HDI | PG | 0.250 | 0.520 | 0.230 | 64.5 | 12.7 |
| 210 | PPG | 1000 | BICH | PG | 0.250 | 0.520 | 0.230 | 9.5 | 4.8 |
| 211 | PPG | 1000 | MDI | PG | 0.250 | 0.520 | 0.230 | 79.8 | 25.7 |
| 212 | PHNAD | 900 | HDI | PG | 0.250 | 0.520 | 0.230 | 125.8 | 17.5 |
| 213 | PHNAD | 900 | BICH | PG | 0.250 | 0.520 | 0.230 | 12.8 | 21.6 |
| 214 | PHNAD | 900 | MDI | PG | 0.250 | 0.520 | 0.230 | 93.3 | 24.7 |
| 215 | PHNAD | 900 | HDI | BD | 0.250 | 0.520 | 0.230 | 21.5 | 23.1 |
| 216 | PHNAD | 900 | BICH | BD | 0.250 | 0.520 | 0.230 | 1.3 | 1.5 |
| 217 | PHNAD | 900 | MDI | BD | 0.250 | 0.520 | 0.230 | 153.0 | 28.5 |

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Table 1. List of screened polymers with monomer composition; average number and standard deviation of the human renal tubular epithelial cell bound to each polymer (average of 4 polymer spots)

**List of abbreviations:**

**Polyol:**

PEG: poly(ethylene glycol)

PPG: poly(propylene glycol)

PTMG: poly(tetramethylene glycol)

PHNAD: poly[1,6-hexanediol/neopentyl glycol-*alt*-(adiptic acid)]diol

PHNGAD: poly[1,6-hexanediol/neopentyl glycol/diethylene glycol-*alt*-(adiptic acid)]diol

**Diisocyanate (Dis.):**

BICH: 1,3-bis(isocynanatomethyl)cyclohexane

MDI: 4,4'-methylenebis(phenylisocyanate)

HDI: 1,6-diisocyanohexane

HMDI: 4,4'-methylenebis(cyclohexylisocyanate)

PDI: 1,4-diisocyanobenzene

TDI: 4-methyl-1,3-phenylene diisocyanate

**Chain Extender (Ext.):**

ED: ethylene diamine

BD: 1,4-butanediol

EG: ethylene glycol

PG: propylene glycol

DMAPD: 3-dimethylamino-1,2-propanediol

DEAPD: 3-diethylamino-1,2-propanediol

DHM: diethyl bis(hydroxymethyl)malonate

NMPD: 2-nitro-2-methyl-1,3-propanediol

OFHD: 2,2,3,3,4,4,5,5-octafluoro-1,6-hexanediol

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## Experimental Procedures

### Substrate Preparation.

The activation of aminoalkylsilane slides (Silane-Prep™; Sigma) was carried out according to the method of M. Beier (*Nucleic Acids Res.* 1999, **27**, 1970-1977). After activation they were treated immediately with 1-octadecylamine (0.20 mmol; 54 mg; Clariant) with 1.0 % v/v diisopropylethylamine in DMF (100 ml) for 4 hours. Finally the alkylated slides were washed with DMF, acetone and dried under a stream of nitrogen. The perfluoroalkylthiol monolayer (B.P. Orner *et al., J. Am. Chem. Soc.*, 2004, **126**, 10808-10809) was formed by immersing the gold coated slide in a 1.0 mM solution of 1H,1H,2H,2H-perfluorodecanethiol (Fluorochem) in methanol for 4 hours, followed by rinsing in methanol and drying under a stream of nitrogen.

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**Array Printing.**

The polymer arrays were fabricated by contact printing (Qarray mini, Genetix) with 16 aQu solid pins (K2785; Genetix; UK) using 1.0 % polymer solutions (w/v) in 1-methyl-2-pyrrolidinone placed into polypropylene 384-wells microplates. The following printing conditions were used, 5 stamping per spot, 200 ms inking time and 10 ms stamping time. The typical spot sizes was 300-320 μm in diameter with spot to spot distances of approximately 1125 μm allowing up to 480 polymers to be printed on a standard 25 x 75 mm slide. In order to assess the reproducibility of the screen, only 120 polymers (from our library of 278 pure poly(urethanes)) were printed in quadruplicate within 2 fields of 16 x 16 spots, while within each field a pattern of 4 x 4 spots was left empty. Once printed, the slides were dried overnight under vacuum at 45 °C and were sterilised by exposure to UV irradiation for 15 minutes.

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**Cell Culture.**

Macroscopically normal kidney tissue was obtained following nephrectomy for renal cell carcinoma. Cortical tissue (2-5 g) was taken into chilled DMEM/Ham's F12 media containing penicillin (200 units/mL), streptomycin (200 mg/mL) and finely chopped using cross blades. The chopped tissue was washed three times with the above media with centrifugation (250 x g) between washes, before being subjected to enzymatic digestion in an orbital incubator for 1 hour at 37 °C using collagenase type IV 0.10 % w/v (Worthington) in PBS. The disaggregated cell mixture was centrifuged at 250 x g for 5 minutes. The resulting cell pellet was resuspended in media and passed sequentially through 70 and 40 μm sieves. After further centrifugation at 250 x g for 5 minutes the cell pellet was resuspended at a density of $10^5$ cells/mL in a fully defined DMEM:Ham's F12 medium containing 2.0 mmol glutamine, 100 iu/ml penicillin, 100 mg/ml streptomycin, 10 ng/ml epithelial growth factor (EGF), 36 ng/ml hydrocortisone, 5.0 μg/ml human insulin, 10 ng/ml prostaglandin-$E_1$ ($PGE_1$), 5.0 ng/ml sodium selanite, 5.0 μg/ml iron loaded transferrin and 5.0 pg/ml tri-iodothyronine ($T_3$) in 25 $cm^2$ filtered culture flasks (Falcon) and incubated at 37 °C in a carbon dioxide (5 %) regulated incubator. Cultures were inspected on a daily basis and the media

changed every 48 hours until grown to confluence. At confluence the cultures were passaged using 0.050 % w/v trypsin, 0.20 % w/v EDTA (Gibco) in PBS and, after washing with PBS-containing trypsin inhibitor, 0.15 % w/v (Sigma) the cells were resuspended ($10^5$ cells/slide) in the growth media on the polymer slides. The growth media was changed every 48 hours and the cells were incubated onto polymer arrays for a total of 5 days. Following washing in PBS, the cells were fixed in 3.7 % w/v p-formaldehyde for 15 min, permeabilised with 0.10 % Triton-X 100 for 2 minutes and washed 3 times in PBS. The slides were blocked with goat serum (1:20 in PBS) and incubated with CAM5-2 (low molecular weight cytokeratin) monoclonal antibody (Cambridge Bioscience) overnight at 4 °C and visualised using an Alexa Fluor® 488 labelled IgG antibody (Molecular Probes) for 30 minutes. The cell nuclei were stained with 0.050 μg/mL Hoechst 33342 (Sigma) for 10 minutes.