Supporting Information

for Adv. Healthcare Mater., DOI: 10.1002/adhm.202002202

Well-defined Nanostructured Biointerfaces: Strengthened Cellular Interaction for Circulating Tumor Cells isolation

Leixiao Yu,* Peng Tang, Chuanxiong Nie, Yong Hou, Rainer Haag*
Supporting Information

**Well-defined Nanostructured Biointerfaces: Strengthened Cellular Interaction for Circulating Tumor Cells Capture**

*Leixiao Yu, * Peng Tang, Chuanxiong Nie, Yong Hou, Rainer Haag*

Dr. Leixiao Yu, Peng Tang, Chuanxiong Nie, Dr. Yong Hou, Prof. Rainer Haag

Institute of Chemistry and Biochemistry, Freie Universität Berlin, Takustr. 3, 14195 Berlin, Germany

Email: leixiaoyu@zedat.fu-berlin.de, haag@chemie.fu-berlin.de
**Figure S1.** Scanning electron microscope images of the self-growing nanoparticles on the substrate surface with the increase of coating time. Scale bar indicates 1 μm.

**Figure S2.** The energy-dispersive X-ray (EDX) spectra of the bare glass, mPG4 coating surface, and the mPG24 coating surface.
Figure S3. The surface roughness of mPG4 surfaces before and after hPG-Cat grafting.

Figure S4. Representative images of MCF7 and HeLa adhesion on the antifouling hPG-mPG4 surface for 3 hours incubation. No cells were found on the surfaces after gently rinsing with PBS buffer. Scale bar indicates 100 μm.
**Figure S5.** The captured MCF7 cells on anti-EpCAM functionalized mPG4 surface with the variation of bioligands density, which is in line with the surficial antibody density. The spiked cell concentration was 4000 cells/mL.

**Figure S6.** Fluorescence microscopy images of the captured cells from MCF7/HeLa mixture on anti-EpCAM functionalized mPG4 surface and mPG4 surface without antibody functionalization and antifouling hPG monolayer coating. The scale bar indicates 100 μm.
Figure S7. The capture purity of cancer cells from artificial patient blood samples on the mPG4 (antibody -) surface and mPG4 (antibody +) surface.
Materials

All chemicals and solvents are reagent or HPLC grade, used as received and purchased from Sigma (Steinheim, Germany) unless stated otherwise. Dialysis was performed in benzoylated cellulose tubes from Sigma-Aldrich (D7884, width: 32 mm, molecular weight cut-off (MWCO) 2000 g·mol⁻¹). The deionized water used was purified using a Millipore water purification system with minimum resistivity of 18.0 MΩ·cm. The hyperbranched polyglycerol with Mn = 5000 g·mol⁻¹ and Mw = 7500 g·mol⁻¹, was polymerized by a one-step ring-opening anionic polymerization (ROAP) as described in the literature.¹ ² The trimethylolpropane (TMP) was used as the initiator.

Synthesis of mPG coating polymer

Poly (allyl glycidyl ether) (PAGE)

Tetraoctylammonium bromide (546 mg, 1 mmol) was heated to melting temperature under a vacuum to remove the trace water. After cooling down to room temperature, 140 mL abs. toluene was added into the Schlenk flask under argon to dissolve this initiator. Then 5.203 mL allyl glycidyl ether (AGE, 5.006 g, 44 mmol) were added to the solution. The polymerization was started by adding the triisobutylaluminum (3.6 mL, 4 mmol) at a constant temperature of 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred overnight. After polymerization, 0.5 mL water was added to quench the polymerization and then the mixture was dried with Na₂SO₄. A
colorless polymer was obtained after the removal of toluene under reduced pressure. Then the polymer was dissolved into Et₂O and centrifuged to remove the residues of initiator and catalyst. The obtained sticky polymer was re-dissolved in tetrahydrofuran (THF) and stirred overnight at room temperature followed by adding 2 mL 1 M NaOH aqueous solution. After removal of the salt and the solvent, the product was further purified by dialysis in dichloromethane and a kind of honey-like compound was obtained. ¹H-NMR (400 MHz, chloroform-d) δ = 5.96 – 5.8 (m, 1H, CH₂OCH₂CH=CH₂), 5.32 – 5.09 (m, 2H, CH₂OCH₂CH=CH₂), 3.97 (d, 2H, CH₂OCH₂CH=CH₂), 3.80 – 3.38 (m, 7H, PG-backbone). $M_n,GPC,\text{THF}=5796 \text{ g/mol}$, $M_w/M_n=1.09$.

Thiol-ene reaction (poly (amino glycidyl ether), PAmGE)

The thiol-ene reactions were carried out by UV irradiation (~1.2 mW/cm², λ = 365 nm) under ambient laboratory conditions. 1 g PAGE and cysteamine hydrochloride (3.98 g, 4 eqv. to allyl groups) was dissolved into 40 mL methanol/THF (1:1). Then 2, 2-dimethoxy-2-phenylacetophenone (Irgacure 651, 2% eqv. to allyl groups) was added and the reaction was conducted under the irradiation of 365 nm...
UV light at room temperature. The reaction was monitored via $^1$H-NMR and completed in 4 hours. Then, 5 mL triethylamine (Et$_3$N) was added into the mixture and stirred at room temperature for 10 hours to remove the hydrochloride. The formed triethylamine hydrochloride salts were filtrated and the filtrate was purified by dialysis in methanol.

$^1$H-NMR (400 MHz, MeOD-d4) δ = 1.78-1.95 (O-CH$_2$-CH$_2$-S, 2H), 2.60-2.73 (O-CH$_2$-CH$_2$-CH$_2$-S, 2H), 2.77-2.9 (S-CH$_2$-CH$_2$-N, 2H), 3.11-3.20 (S-CH$_2$-CH$_2$-N, 2H), 3.8-3.43 (PG backbone), (O-CH$_2$-CH$_2$-, 2H).

*Catechol coupling (mPG)*

The mussel-inspired coating polymer was prepared by the amide-coupling reaction between the PAmGE polymer and the 3,4-dihydroxyhydrocinnamic acid (DHHA). To prevent the Michael addition and dopa-dopa coupling during the reaction, an acidic buffer was used and the grafting density of catechol groups was precisely controlled by the equivalence ratio of amino groups to carboxyl groups. PAmGE and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI, 3 eqv. to
amino groups) were dissolved into pH 4.8 MES aqueous buffer/methanol (1/1, v/v) mixture solvent. Then 3,4-dihydroxyhydrocinnamic acid (3 eqv. to amino groups) was added into the mixture and gently stirred overnight at room temperature. The product was purified by dialysis and the grafting density of catechol groups was calculated by the $^1$H-NMR, which was 70%.

$^1$H-NMR (400 MHz, MeOD-d4) δ = 1.67-1.95 (O-CH$_2$-CH$_2$-CH$_2$-S, 2H), 2.34-2.68 (O-CH$_2$-CH$_2$-CH$_2$-S, 2H), (S-CH$_2$-CH$_2$-N, 2H), (CONH-CH$_2$-CH$_2$-Ar, 2H); 2.69-2.94 (S-CH$_2$-CH$_2$-N, 2H), (CONH-CH$_2$-CH$_2$-Ar, 2H); 3.8-3.43 (PG backbone), (O-CH$_2$-CH$_2$, 2H).

Synthesis of hPG-Cat5 polymer
**O-Mesylpolyglycerol (hPG-OMs)**

The reaction was carried out under an argon atmosphere and exclusion of water. Hyperbranched polyglycerol (10.0 g, 135 mmol OH-groups) was dissolved in abs. dimethylformamide (DMF, 50 mL) in a Schlenk flask and cooled down to 0 °C in an ice bath. A solution of MsCl (8.1 mmol) in abs. DMF (10 mL) was added dropwise into the cooled hPG solution. After stirring under argon atmosphere for 16 h, the solvent was removed by rotary evaporation, and the residue was re-dissolved and dialyzed in MeOH for 24 h to give a brown honey-like product.

$^1$H-NMR (400 MHz; D$_2$O): $\delta = 4.14$-3.39 (m, PG-backbone); and 3.32-3.28 (s, Ms); 1.43-1.41 (m, C$\text{CH}_2$CH$_3$ of starter); 0.89 (t, C$\text{CH}_2$CH$_3$, of starter) ppm.

**Polyglycerolazide (hPG-N$_3$)**

O-mesylpolyglycerol was dissolved in DMF upon ultra-sonification in a flask with a reflux condenser. After the addition of NaN$_3$ (3 eq. to -OMs groups), the resulting suspension was heated to 80 °C for 3 days. After cooling down to room temperature, the mixture was filtrated by celite to remove away
the excess NaN$_3$. The filtrate was concentrated and the residue was further purified by dialysis in H$_2$O for 24 h to give a brown paste-like compound.

$^1$H-NMR (400 MHz; MeOD): $\delta = 4.2-3.45$ (m, PG-backbone); 1.43-1.41 (m, CCH$_2$CH$_3$ of starter); 0.89 (t, CCH$_2$CH$_3$, of starter) ppm. IR : $\nu$ (cm$^{-1}$): 3365, 2869, 2096 (N$_3$), 1648, 1453, 1272, 1066, 930, 866.

Polyglycerolamine (hPG-NH$_2$)

Polyglycerolamine was synthesized by a Staudinger reaction. Polyglycerolazide and 1.2 eq of PPh$_3$ were dissolved in a mixture of THF/H$_2$O (3/1, v/v). After 2 days of reacting at 45 °C, THF in the mixture was removed by evaporation. The byproduct PPh$_3$O, which cannot be dissolved in H$_2$O, was removed by filtration. And the filtrate was then purified by dialysis in MeOH for 20h.

$^1$H-NMR (400 MHz; D$_2$O): $\delta = 4.12-3.4$ (m, PG-backbone); 1.41-1.39 (m, 2H, CCH$_2$CH$_3$ of starter); 0.90 (t, 3 H, CCH$_2$CH$_3$, of starter) ppm. IR : $\nu$ (cm$^{-1}$): 3349, 2864, 1659, 1597, 1456, 1322, 1069, 1024, 930, 863.

Catecholic polyglycerol (hPG-Cat5)

The polyglycerolamine (with 5% amino groups) and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI, 3 eqv. to amino groups) were dissolved into pH 4.8 MES aqueous buffer/methanol (1/1, v/v) mixture solvent. Then 3,4-dihydroxyhydrocinnamic acid (3 eqv. to amino groups) was added into the mixture and gently stirred overnight at room temperature. The product was purified by dialysis and the grafting density of catechol groups was calculated by the $^1$H-NMR.

$^1$H-NMR (400 MHz; MeOD): $\delta = 6.8-6.54$ (Ar-H, 3H from catechol group.); 4.0-3.40 (PG backbone); 2.76 (2H, COCH$_2$CH$_2$C); 2.47 (2H, COCH$_2$CH$_2$C); 1.41-1.39 (2H, CCH$_2$CH$_3$ of starter); 0.90 (3 H, CCH$_2$CH$_3$, of starter) ppm.

Biotin-phenylboronic acid bioligand (Biotin-PBA)
N-Succinimidyl 9-(biotinamido)-4,7-dioxanonanoate (Biotin-EG2-NHS) (50 mg, 0.1 mmol) and 4-aminophenylboronic acid hydrochloride (16.5 mg, 0.095 mmol) were dissolved in abs. dry DMF in a 10 mL flask. Then 20 μL Et₃N was added and the mixture was stirred at room temperature for 4 h. After the reaction, the solvent was removed under high vacuum and the product was further purified via HPLC.

Mussel-inspired mPG coating

Nanostructured mPG coatings were prepared via a dip-coating method. Generally, freshly cleaned glass slides were immersed into a solution of 1 mg/mL catecholic polyglycerol in a mixture of MeOH and 3-(N-morpholino)-propanesulfonic acid (MOPS, 0.1M, pH 8.6) buffer (v/v 3:1) at room temperature for 20 hours. The depth of the solution was 1 cm. After that, the coated slides were thoroughly rinsed with methanol and Milli-Q water, dried by N₂ stream, and fully dried in a vacuum.
hPG-Cat coating

The prepared mPG coating slides were dipped into the coating solution of hPG-Cat5 (1mg/mL) in pH 8.6 MOPS buffer (0.1 M) at room temperature for 12 h. Under basic conditions, the catechol groups from hPG-Cat were oxidized into quinone and further reacted with the present amino groups from mPG coating via the Michael addition or coupled with catechol groups on the mPG surface via the dopa-quinone coupling. After that, the slides were thoroughly rinsed with methanol, water, and then dried by an N₂ stream.

Bioligands immobilization via the diols-boronic ester dynamic bonding

Phenylboronic acid-functionalized biotin was immobilized onto hPG-Cat coated mPG surface via the dynamic diols-boronic ester bonding. We immersed the hPG-mPG coating surface into PBA-Biotin solution (1 mM, in methanol) and kept them at room temperature for 4 hours. Then the
functionalized slides were taken out and rinsed with methanol and water to remove unbonded biotin-PBA, and then dried with an N₂ stream.

**Biomarkers conjugation**

The immobilization of anti-EpCAM onto biotin-mPG coating was realized via the high bio-specific biotin-avidin noncovalent interaction. The biotin-mPG coating surfaces were incubated into 1 mg/mL NeutrAvidin (Thermo Fisher Scientific, USA) solution in DPBS at 37 °C for 1 h and then thoroughly rinsed with DPBS to remove excess NeutrAvidin. The obtained NeutrAvidin-mPG surface was kept in DPBS solution at 4 °C until ready for use. Before cell experiments, biotinylated anti-EpCAM (12.5 μg/mL in DPBS, containing 1 wt% BSA) were readily conjugated onto NeutrAvidin-mPG surfaces at 37 °C (incubation time: 1 h).

**Quartz Crystal Microbalance (QCM) with Dissipation**

Quartz crystal microbalance (QCM, Q-Sense E1, Sweden) with dissipation was used to study the adsorption on the surfaces. QCM with dissipation monitors the changes in resonance frequency (Δf) and dissipation (ΔD) of a piezoelectric quartz crystal as a function of time. The f and D were recorded at the fundamental frequency (4.95 MHz) and its 3rd, 5th, 7th, 9th, 11th, and 13th overtones. Only the 3rd overtone was shown in the sensorgrams.

For the online coating of hPG-Cat5, the mPG4 coating sensor chip was inserted into the flow chamber (QFM 401, QSense, Sweden, internal volume of 40 μL) and incubated in pH 8.5 MOPS buffer.
with a flow rate of 0.1 mL/min at 25 °C until the equilibration of the baseline. A solution of hPG-Cat5 (1 mg/mL in pH 8.6 MOPS buffer) was pumped into the flow chamber (0.1 mL/min). After 1 h of dynamic online adsorption, the flow chamber was alternately rinsed with pH 8.6 MOPS buffer and Milli-Q water (0.1 mL/min). The Sauerbrey equation was used to calculate the mass of the adsorbates (\( \Delta m = C \times \Delta f \), where \( \Delta m \) is the change in mass, \( C \) is the mass sensitivity constant of the quartz crystal (-17.7 ng·cm\(^{-2}\)·Hz\(^{-1}\)), and \( \Delta f \) is the overtone-normalized frequency change).

The in-situ immobilization of the PBA-biotin onto the coating surface was also monitored by QCM. hPG-Cat5 coated mPG4 coating sensors were incubated in methanol. After baseline equilibrium, the PBA-biotin in methanol (1mM) was pumped into the flow chamber. After 30 min, the sensor was rinsed with DPBS buffer again until the baseline equilibrated again.

A similar protocol was used to study the conjugation of antibodies onto the coating surface. Biotinylated mPG coating sensors were incubated in pH 7.4 DPBS buffer. After baseline equilibration, the NeutrAvidin (1 mg/mL in DPBS buffer) was pumped into the flow chamber. After 30 min, the sensor was rinsed with DPBS buffer for 15 min. Then freshly prepared antibody solution (anti-EpCAM, 12.5 \( \mu \)g/mL in DPBS buffer with 1 wt % BSA) flowed into the NeutrAvidin modified sensor surface for 30 min. The sensor was rinsed with DPBS buffer again for another 15 min. The flow rate used for all experiments was 0.05 mL/min, and the temperature was 25 °C. The Voigt model for viscoelastic layers was used to calculate the mass of adsorbed proteins with the same method as we reported.

**Water Contact Angle (WCA)**

Water contact angle was measured using a contact angle goniometer system (DataPhysics Instruments, Germany). A droplet of 2 \( \mu \)L Milli-Q water was placed on the substrate with the sessile drop method and equilibrated for 10 s at room temperature. Fifteen parts of five parallel coating slides were measured and averaged to get a reliable value.
Atomic Force Microscopy (AFM)

The AFM results were recorded by a MultiMode Nanoscope V scanning probe microscopy (SPM) system (Bruker, USA) in the air to investigate the surficial morphology and structure of the resulting coating surfaces. The commercially available AFM cantilever tips (PPP-NCLR-20 probes from NanoAndMore, GmbH) with a force constant of ~ 48 N/m and resonance vibration frequency of ~ 330 kHz were used, and the scanning rate was set at 0.8 Hz. The AFM mode, Peak Force QNM, was used for better controlling the force with which the tip interacts with the surface.

Scanning electron microscope (SEM) for coating morphology

The surface morphology of the coatings was investigated with the scanning electron microscope (SEM, Hitachi SU8030, Japan) at an accelerating voltage (Vacc) of 20 kV, a current of 10 μA, and a working distance (WD) of around 8.3 mm. The samples were dried under high vacuum and coated with an 8-10 nm gold layer by using a sputter coater (Emscope SC 500, Quorum Technologies, UK) for 20 s at 30 mA, 10⁻¹ Torr (1.3 mbar) in an argon atmosphere.

SEM images for cells on the mPG coating surface

After 3h culturing on mPG coating surface in RPMI 1640 medium, the cells were gently rinsed with DPBS, fixed with glutaraldehyde solution (2.5 wt % in DPBS) for 60 min at room temperature, and thoroughly removed the glutaraldehyde with DPBS. Cells were then dehydrated by mixtures of ethanol and DPBS with different ratios (30/70, 50/50, 70/30, 85/15, 95/5, 100/0, and 30 min for each) at room temperature. Residue water in cells was further thoroughly removed by
hexamethyldisilazane/ethanol (50/50, v/v, 30 min) and hexamethyldisilazane (30 min). After pipetting out hexamethyldisilazane, the coating slides with cells were put into a hood overnight at room temperature and then a desiccator in vacuum for drying. All samples were sputtered a thin gold layer (sputtering for 60 s, Emscope SC 500, Quorum Technologies, UK) and imaged with the scanning electron microscope (Hitachi SU8030, high voltage on 10 kV).

**Cell Culture**

Roswell Park Memorial Institute RPMI-1640 culture medium (Gibco) was supplemented with 10% (v/v) fetal bovine serum (FBS) (Gibco), and 1% (v/v) of penicillin-streptomycin solution (Gibco). Human breast cancer cell lines (MCF7, T47D, MDA-MB-231), human lung cancer cell line (A549) (DSMZ no.: 115), and human cervical cancer cell lines (HeLa) (DSMZ no.: ACC 57) were obtained from the American Type Culture Collection (ATCC). GFP⁺-HeLa cells were derived from the HeLa cell line (ATCC; CCl-2), which were stably transfected with an expression unit for a destabilized enhanced green fluorescent protein (d2EGFP, Clontech, Palo Alto, USA). All the cancer cell lines were routinely cultured at 37 °C with 5% CO₂ in a 24-well plate in an air atmosphere.

**Cancer Cell Capture**

Before the capture experiment, the cancer cells were pre-stained with CellTraceTM violet dye (Thermo Fisher Scientific, Waltham, MA, USA) for 20 min by using the standard protocol. Stained cells were spiked at a density of 3000 cells/mL on anti-EpCAM functionalized mPG coating surface in serum-free RPMI-1640 culture medium at a concentration of 3000 cells/mL and incubated at 37 °C for 60 min. Then the mPG surfaces were gently rinsed with DPBS three times and observed by the
fluorescence microscope (Zeiss Axio Observer Z1, Germany). The corresponding capture efficiency was calculated via the previously reported method.\(^3\)

**Cancer cell capture from artificial patient blood samples**

Human blood samples were collected in Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) from healthy donors. The blood samples were processed or lysed within 2 h after the blood draw. Briefly, the blood was lysed using red blood cell (RBC) lysing buffer (1 g potassium hydrogen carbonate, 18.3 g ammonium chloride, and 0.3 g anticoagulant ethylenediaminetetraacetic acid in 1 L \(\text{H}_2\text{O}, \text{pH} = 7.5\)). Different numbers of CellTrace\textsuperscript{TM} violet pre-stained cancer cell lines were then spiked in healthy whole blood or lysed blood to prepare artificial patient blood samples. After incubated in the artificial patient blood samples for 60 min at 37 °C antibody-functionalized mPG surfaces were rinsed with DPBS and observed by fluorescence microscope (Zeiss Axio Observer Z1, Germany).

**Cell release and re-culture**

Due to the dynamic diols-boronic ester bonding, the captured cancer cells on mPG surfaces can be released by adding D-sorbitol. The released cells were collected and seeded in a new cell culture plate for re-culturing. Cell Counting Kit-8 (CCK8) (Dojindo Molecular Technologies, Inc., Rockville, USA) assays were performed to test the proliferation of released cells and determine cell numbers based upon reaction product optical density (OD, at 450 nm) value. Microscope (Zeiss Axio Observer Z1, Germany) was used to observe and image the cells.
Immunofluorescence staining

Cells were washed twice with DPBS before fixation with 4% paraformaldehyde at room temperature for 15 minutes. Samples were then washed 3 times with ice-cold PBS. Cells were permeabilized with 0.25% (v/v) Triton-X 100 in PBS for 10 minutes at room temperature, then washed 3 times with PBS. Non-specific antibody binding was blocked by incubating samples with 1% (w/v) bovine serum albumin (BSA) in PBST (0.1% v/v Triton-X 100 in PBS) at room temperature for 45 minutes. Next, samples were washed briefly with PBST and incubated with dye-labeled primary antibodies overnight at 4 °C. Following primary antibody incubation, samples were washed twice with PBST and 3 times with PBS. Samples were then incubated with DAPI for 60 minutes at room temperature for one hour, followed by washing twice with PBST and 3 times with PBS. Immunofluorescence images were acquired on a Zeiss Axio Observer Z1 microscope or a Leica SP8 confocal microscope.

Statistics

Statistical analysis was performed in GraphPad Prism 7/8. One-way ANOVA followed by post hoc Tukey’s multiple comparisons test was carried out for group differences. Significance is indicated by p < 0.05 (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). All results are displayed as mean ± standard deviation. The sample size is indicated within the corresponding figure legends. All data are presented as mean ± standard deviation.

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