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UV- and Thermally Triggered Ring-Opening Metathesis Polymerization for the Spatially Resolved Functionalization of Polymeric Monolithic Devices

Claudia Ernst,[1] Christian Elsner,[1] Andrea Prager,[1] Bettina Scheibitz,[1] Michael R. Buchmeiser[2,3]

[1]*Leibniz-Institut für Oberflächenmodifizierung e.V. (IOM), Permoserstraße 15, D-04318 Leipzig, Germany*
[2]*Lehrstuhl für Makromolekulare Stoffe und Faserchemie, Institut für Polymerchemie, Universität Stuttgart, Pfaffenwaldring 55, D-70550 Stuttgart, Germany*
[3]*Institut für Textilchemie und Chemiefasern, Körschtalstr. 26, D-73770 Denkendorf, Germany*

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ABSTRACT: Porous polymeric monolithic supports were prepared via electron beam-triggered free radical polymerization using a mixture of ethyl methacrylate and trimethylolpropane triacrylate in 2-propanol, 1-dodecanol and toluene. Bicyclo[2.2.1]hept-5-en-2-ylmethyl acrylate (1) was grafted onto these monolithic supports in a spatially resolved way with the aid of masks using both electron beam- (EB) and UV-triggered free radical polymerization. The thus immobilized norborn-2-ene-containing graft polymers were further treated with the 2nd-generation Grubbs initiator, i.e., $RuCl_2(PCy_3)(IMesH_2)(CHPh)$ (4) ($IMesH_2$ = 1,3-dimesitylimidazolin-2-ylidene), and then reacted with bicyclo[2.2.1]hept-5-en-2-ylmethyl pyrene-1-carboxylate (2). Alternatively, monoliths completely grafted with poly-**1** were surface grafted with **2** in a spatially resolved way in the presence of a latent, UV-triggerable precatalyst, i.e., $[Ru(IMesH_2)(CF_3COO)(t\text{-}BuCN)_4^+\ CF_3COO^-]$ (5). Finally, to demonstrate the utility of this chemistry, a 2nd-generation Grubbs initiator-based approach was used to prepare a trypsin-functionalized monolith-containing chip device that allowed for the online digestion of *N*-α-benzoyl-L-arginineethylester hydrochloride. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 2551–2558, 2011

Key words: monoliths; ring-opening metathesis polymerization; ruthenium; surface grafting; UV

INTRODUCTION

Polymeric monolithic materials have gained a strong position in separation science.[1–8] In particular, monoliths for small-scale separations, i.e., monolithic μ-HPLC columns have moved into the center of interest.[9] In parallel, with the synthetic methodologies for monolithic devices becoming both more and more sophisticated and robust, chip devices based on monolithic materials are more frequently used.[10–18] Because they may contain more than one inlet and outlet, respectively, such chips allow not only for the separation of compounds but also for one or more preceding inline reaction steps. This is usually accomplished with the aid of monolith-immobilized (bio-)catalysts.[11,18] So far, the area of monolithic materials has been dominated by thermally or UV-triggered free radical polymerization.[19] However, during the last 10 years, we contributed to that area by developing both ring-opening metathesis polymerization (ROMP)[20,21] and electron-beam-triggered, free radical polymerization approaches.[22–27] Here, we describe our latest accomplishments in the fabrication of polymeric monolithic chip devices and their spatially resolved derivatization using both thermally triggered and UV-triggered ROMP. Finally, a simple application in the area of on-chip reaction/analysis is presented.

EXPERIMENTAL

5-Norbornene-2-methanol (98%, *endo*/*exo*-mixture, ChemService), 1-pyrenecarboxylic acid (97%), acrylic acid chloride (97%), trimethylolpropane triacrylate (TMPTA, techn. grade), fluorescein isothiocyanate isomer I (≥90%), trypsin from bovine pancreas, ribonuclease A from bovine pancreas, albumin from bovine serum, cytochrome c from bovine milk, $RuCl_2(PCy_3)(IMesH_2)(CHPh)$ (**4**, $IMesH_2$ =

Additional Supporting Information may be found in the online version of this article.

Correspondence to: M. R. Buchmeiser (michael.buchmeiser@ipoc.uni-stuttgart.de).

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1,3-dimesitylimidazolin-2-ylidene), ethyl vinyl ether (EVE), and water were from Sigma-Aldrich (Taufkirchen, Germany). *N*-hydroxysuccinimide (≥97%), ethyl methacrylate (EMA, ≥99%), and 2-propanol (99.8%) were purchased from Fluka (Biuchs, Switzerland). 3-(Trimethoxysilyl)propyl methacrylate and 1-dodecanol (98%) from Alfa Aesar (Karlsruhe, Germany), Lucirin® TPO-L (BASF), HEPES pufferan®, Roti®-stock 10× PBS, HEPES buffer pH 8.0: 0.1*M* HEPES, 0.2*M* NaCl, 0.02*M* $CaCl_2$, and the HPLC-grade solvents rotisolv® methanol, ethanol, acetonitrile from Carl Roth (Karlsruhe, Germany) were used without any further purification. $CHCl_3$ was dried over CaH_2, toluene and CH_2Cl_2 were dried by an MBraun SPS system (MBraun, Garching, Germany). Lysozyme from chicken egg was from Carl Roth. Fused silica capillary columns (0.20 mm I.D., 0.34 mm O.D.) were from Agilent Technologies and UV transparent capillary columns (0.10 mm I.D.) were from Polymicro Technologies (Phoenix, Arizona). Microreactor chips FC_R150.676.2 for functionalization experiments and R150.332.X for protein separation experiments (Micronit, internal volume 13 μL and 6 μL, respectively, channel width 150 μm, channel depth 150 μm (http://www.micronit.com/en/products/fluidic_chips/microreactor_chips.php, Enschede, The Netherlands) were used (Supporting Information). Nuts and ferrules (F-123HX and *N*-123-03X) were from Upchurch Scientific (Shipley, UK). GC-measurements were carried out on a GC 2010 and a GC-17A, respectively, (both from Shimadzu, Duisburg, Germany). The column oven temperature was 70°C; the injection temperature was set to 150°C, the ion source temperature was set to 200°C, the interface temperature was set to 300°C. A Vector 22 IR-Spectrometer running under OPUS 6.5 software (all Bruker, Karlsruhe, Germany) was used for IR measurements. NMR measurements were performed on a 600 MHz AVANCE II 600 NMR-spectrometer (Bruker). For microscopy, a Leica DMLM, equipped with a 100 W Hg-lamp and a DFC 350 FX camera, was used. Electron microscopy was carried out on a field emission gun-REM (Zeiss Ultra 55 from Carl Zeiss SMT, Oberkochen, Germany). A 10 MeV electron accelerator (ELEKTRONIKA, Toriy Company, Moscow, Russia) was used for electron beam curing. A pulse frequency of 50 Hz and a pulse length of 4 μs were applied using a scanning window 40 cm in width (scanning frequency 1 Hz) and a movable probe table. For referencing, the total dose on graphite was measured by calorimetry without any correction for the irradiated material. For monolith synthesis, the total dose was applied in ~3 kGy steps over a time of 15 min to minimize the temperature raise. An Acclaim-PepMap100 (C18, 3 μm, 100 Å, 300 μm I.D. × 15 cm, Dionex, Idstein, Germany), a syringe pump (World Precision Instruments, Sarasota, FL), and a medium pressure M 400 U 1 Hg-lamp (IST GmbH, Nürtingen, Germany, 120 W/cm, O_2-content < 100 ppm, dose 670 mJ/cm^2) were used. Finally, an Ultimate 3000 HPLC System equipped with a HPG-3400*M* pump, a WPS-3000 SL autosampler (all Dionex), and a Foxy Jr.® fraction collector, (Teledyne ISCO) as well as an Ultimate 3000 HPLC System equipped with an LPG-3400*M* pump, a WPS-3000 autosampler, an FLM-3300 flow manager, a VWD-3400 detector, an SRD-3000 degasser, and a CAP 100 flow splitter (all Dionex) were used. Bicyclo[2.2.1]hept-5-en-2-ylmethyl acrylate (**1**),[22] 7-oxanorborn-5-ene-2-carboxylic acid chloride,[28] and the latent ROMP initiator [Ru(OOCCF$_3$)(IMesH$_2$)(*t*-BuCN)$_4^+$ CF$_3$COO$^-$] (**5**)[29] were prepared according to the literature.

Bicyclo[2.2.1]hept-5-en-2-ylmethyl pyrene-1-carboxylate (2)

1-Pyrenecarboxylic acid (500 mg, 2.0 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (380 mg, 2.4 mmol), and 4-(dimethylamino)-pyridin (250 mg, 2.0 mmol) were dissolved in 50 mL of DMF. 5-Norbornene-2-methanol (218 mg, 1.75 mmol), dissolved in 2 mL of DMF, was added and the mixture was stirred for 72 h. Then, the solvent was removed *in vacuo* and the residue was dissolved in diethyl ether. The organic phase was subsequently washed with a 5 wt % solution of citric acid, then with a 5 wt % solution of $NaHCO_3$, and then with water. The organic solvent was removed *in vacuo*. The crude product was purified by column chromatography (*n*-pentane/EtOAc 90/10 v/v). Yield: 210 mg (0.60 mmol, 30%). ^{1}H-NMR ($CDCl_3$, 600 MHz): *endo*-diastereomer: δ [ppm] = 9.28–9.26 (1 H, d, $^3J_{H,H}$ = 9.3 Hz, **H-9**), 8.65–8.64 (1 H, d, $^3J_{H,H}$ = 8.1 Hz, **H-10**), 8.27–8.04 (7 × 1 H, m, **Ar-H**), 6.28–6.26 (1 H, dd, $^3J_{H,H}$ = 3.1/5.5 Hz, **H-5**), 6.14–6.12 (1 H, dd, $^3J_{H,H}$ = 2.9/5.5 Hz, **H-6**), 4.33–4.31 (1 H, dd, $^2J_{H,H}$ = 10.6 Hz, $^3J_{H,H}$ = 6.7 Hz, **H-8a**), 4.10 (1 H, dd, **H-8b**), 3.12 (1 H, s, **H-1**), 2.92 (1 H, s, **H-4**), 2.73–2.68 (1 H, m, **H-2**), 2.03–1.98 (1 H, m, **H-3a *exo***), 1.56–1.37(2 H, m, **H-7a,b**), 0.80–0.76 (1 H, m, **H-3b *endo***); *exo*-diastereomer: δ [ppm] = 9.29–9.27 (0.3 H, d, $^3J_{H,H}$ = 9.3 Hz, **H-9**), 8.66–8.65 (0.3 H, d, $^3J_{H,H}$ = 8.1 Hz, **H-10**), 8.27–8.04 (7 × 0.3 H, m, **Ar-H**), 6.21–6.19 (0.3 H, dd, $^3J_{H,H}$ = 3.2/5.3 Hz, **H-5**), 6.17–6.15 (0.3 H, dd, $^3J_{H,H}$ = 2.9/5.5 Hz **H-6**), 4.62–4.59 (0.3 H, dd, $^2J_{H,H}$ = 10.8 Hz, $^3J_{H,H}$ = 6.7 Hz, **H-8a**), 4.44–4.41 (0.3 H, dd, $^2J_{H,H}$ = 10.4 Hz, $^3J_{H,H}$ = 9.5 Hz **H-8b**), 2.94 (2 × 0.3 H, s, **H-1,4**), 2.03–1.98 (0.3 H, m, **H-2**), 1.56–1.37 (4 × 0.3 H, m, **H-3a,b**, **H-7a,b**); ^{13}C{^{1}H}-NMR, ^{13}C-DEPT ($CDCl_3$, 150 MHz): *endo*-diastereomer: δ [ppm] = 168.2 (**C$_q$**=O), 137.9 (**C-5**), 132.4 (**C-6**), 68.8 (**C-8**), 49.7 (**C-7**), 44.3 (**C-1**), 42.5 (**C-4**), 38.2 (**C-2**), 29.3 (**C-3**); *exo*-diastereomer: δ [ppm] = 168.2 (**C$_q$**=O), 137.2 (**C-6**), 136.5 (**C-5**), 69.5 (**C-8**), 45.3 (**C-7**), 44.1 (**C-1**), 41.9 (**C-4**), 38.4 (**C-2**), 30.0 (**C-3**); *endo/exo*-diastereromer: 129.7, 129.7, 129.5, 129.5, 128.5, 128.5,

127.3, 126.4, 126.4, 126.3, 126.3, 126.2, 125.1, 125.0, 124.3, 134.4, 134.3, 131.2, 131.2, 131.1, 130.5, 125.0, 124.4, 124.4, 124.1, 124.0. IR (ATR Mode): 3051 (m) $\nu_{(CH\ sp2)}$, 2958 (s), 2939 (s) $\nu_{(CH2\ sp3)}$, 2866 (m) $\nu_{(CH\ sp3)}$, 1702 (s) $\nu_{(C=O)}$, 1596 (m) $\nu_{(C=C)}$, 1387 (m), 1327 (m), 1250 (s), 1230 (s) $\nu_{(C-O)}$, 1196 (m), 1146 (s), 1133 (s), 1043 (s), 849 (s), 710 (s), 631 (s).

7-Oxabicyclo[2.2.1]hept-5-ene-2-carboxylic acid *N*-hydroxysuccinimide ester (3)

7-Oxabicyclo[2.2.1]hept-5-ene-2-carboxylic acid chloride (164 mg, 1.00 mmol) was slowly added to a solution of *N*-hydroxysuccinimide (138 mg, 1.2 mmol) and triethylamine (121 mg, 1.2 mmol) dissolved in 15 mL of dry CH_2Cl_2. The mixture was stirred for 4 h and then washed twice with cold water. Finally, the organic layer was dried over $MgSO_4$ and all volatiles were removed *in vacuo*. The crude product (160 mg, 67%) was washed with cold diethyl ether and then purified by column chromatography (silica gel 60, 35–70 μm, ethyl acetate). The target compound was obtained as a white, crystalline compound **130** mg (0.55 mmol, 55%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 6.47–6.45 (1H, dd, $^3J_{H,H}$ = 5.8/1.5 Hz, **H-6**), 6.40–6.39 (1H, dd, $^3J_{H,H}$ = 5.7/1.4 Hz, **H-5**), 5.34 (1H, s, **H-1**), 5.16–5.15 (1H, d, $^3J_{H,H}$ = 4.4 Hz, **H-4**), 2.84–2.83 (4H, bd, $^2J_{H,H}$ = 9.8 Hz, **H-10**), 2.72–2.70 (1H, dd, $^3J_{H,H}$ = 8.5/4.0 Hz, **H-2**), 2.27–2.24 (1H, dt, $^2J_{H,H}$ = 11.6 Hz, $^3J_{H,H}$ = 4.3 Hz, **H-3a**), 1.70–1.73 (1H, dd, $^2J_{H,H}$ = 11.7 Hz, $^3J_{H,H}$ = 8.5 Hz, **H-3b**); ^{13}C{^{1}H}-NMR, ^{13}C-DEPT ($CDCl_3$, 150 MHz): δ [ppm] = 169.6 (O=**C-8**), 169.3 (O=**C-9**), 137.6 (**C-6**), 132.0 (**C-5**), 81.1 (**C-1**), 78.2 (**C-4**), 40.4 (**C-2**), 30.0 (H_2**C-3**), 25.7 (H_2**C-10**). IR (ATR-Mode): $\tilde{\nu}$ [cm^{-1}] = 3100–3000 (w) $\nu_{(CH\ sp2)}$, 2949 (m) $\nu_{(CH,\ CH2\ sp3)}$, 1807 (s), 1778 (s) ν(C=O), 1730 (s) ν(C=O), 1571 (m) $\nu_{(C=C)}$, 1427 (m), 1361 (s), 1315 (m), 1273 (m), 1200 (s), 1155 (m), 1140, 1105, 1061, 1047, 1020, 995, 947, 895, 872, 850, 804, 737, 706, 642.

Pretreatment of capillary columns and chips

The empty capillary columns and chips, respectively, were subsequently washed with 4 mL of ethanol, 4 mL of distilled H_2O, 4 mL of 1*M* NaOH, and finally with 8 mL of distilled H_2O using a syringe pump. After flushing with air, they were dried at 40°C for 1 h *in vacuo*. The devices were then filled with a 50 wt % solution of 3-(trimethoxysilyl)propyl methacrylate (MEMO) in toluene, the ends of the device were closed with septa, and the device was heated to 60°C for 16 h. Finally, the device was flushed with 4 mL of toluene, 4 mL of acetone, and then with air and dried at 40°C *in vacuo*. Generally, a flow rate of 0.8 mL/min, 0.2 mL/min, and 0.1 mL/min, respectively, was used for 200 μm, 100 μm capillary columns and for the chip.

Synthesis of monoliths

Solutions based on EMA/TMPTA/2-PrOH/1-dodecanol/toluene (15/15/30/30/10) were twice subjected to a freeze-pump-thaw cycle. A capillary column 17 cm in length and a chip, respectively, were filled with the solution, closed with septa and subject to electron beam treatment using a 10 MeV electron accelerator. A total dose of 22 kGy was applied. Then, the monoliths were flushed with acetonitrile for at least 1 h (flow rate = 5 μL/min).

EB-based spatially resolved immobilization of 1 on monoliths

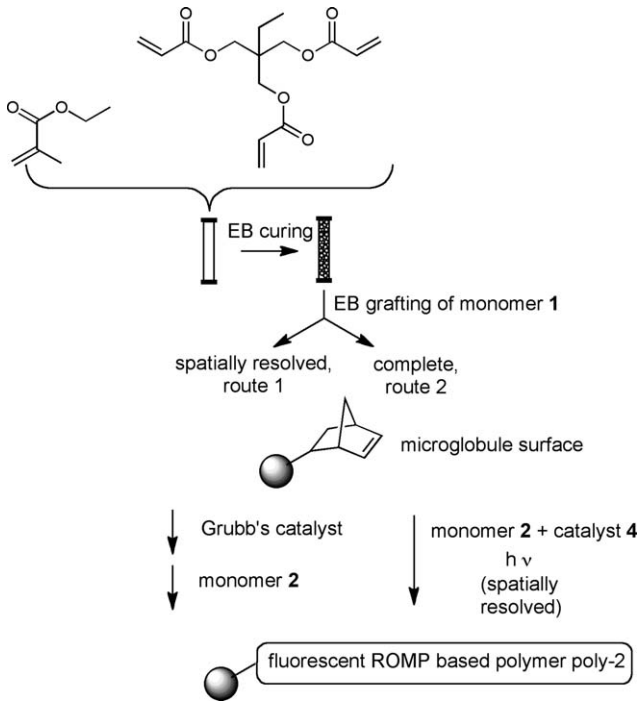
Monoliths were prepared as described above. The monoliths were flushed with ethanol, and then a 5 wt % solution of **1** in ethanol was passed through the device for 20 min. Complete filling of the device was checked by monitoring the effluent with TLC. The device was closed and subject to EB irradiation applying 22 kGy. For the spatially resolved immobilization of **1**, the desired parts of the capillary were covered with either Pb or graphite plates. Finally, the column was flushed with ethanol.

UV-based spatially resolved immobilization of 1 on monoliths

The monoliths were prepared as described above and flushed with ethanol, then a solution of 5 wt % of **1** and 0.15 wt % of lucirin® TPO-L in ethanol was passed through the device for 20 min. The complete filling of the device was checked by monitoring the effluent via thin layer chromatography (TLC). The device was closed and subject to UV-irradiation applying a dose of 3 × 670 mJ/cm^2. For the spatially resolved immobilization of **1**, the desired parts of the capillary were covered with aluminum foil. Finally, the column was flushed with ethanol.

ROMP-based spatially resolved immobilization of 2 on monoliths using $RuCl_2(PCy_3)(IMesH_2)(CHPh)$ (4)

A monolith surface grafted with poly-**1** in a spatially resolved way was used. The column was flushed with dry $CHCl_3$ for 30 min. Then, a solution of $RuCl_2(PCy_3)(IMesH_2)(CHPh)$ (**4**, 4 mg/mL in $CHCl_3$) was passed through the column for 20 min. The ends of the device were closed with septa and the device was stored at room temperature for 1 h. Then, the column was flushed with dry $CHCl_3$ for 15 min to remove any unreacted initiator. Finally, the columns were filled with a solution of **2** in $CHCl_3$ (10 mg/mL), closed, and reacted for 1 h. Any unreacted monomer was removed by flushing the column with dry $CHCl_3$ for 1 h.



Scheme 1 Different ROMP-based techniques for the spatially resolved immobilization of the fluorescence marker **2** on a monolith.

ROMP-based spatially resolved immobilization of 2 on monoliths using a latent UV-triggerable ROMP precatalyst (5)

A monolith completely surface grafted with poly-**1** was used. The column was flushed with dry $CHCl_3$ for 30 min. Then a mixture of the latent UV-triggerable precatalyst (4 mg/mL) and **2** (10 mg/mL) in $CHCl_3$ was flushed through the column for 20 min. The ends of the device were closed with septa and the device was exposed to UV-light ($\lambda = 254$ nm, 3.5 mW/cm^2, 60 min = 12.6 J/cm^2) covering the desired parts with aluminum foil. Any unreacted monomer as well as soluble oligomer/polymer that formed was removed by flushing the column with dry $CHCl_3$ overnight to remove any soluble polymer fractions.

Spatially resolved immobilization of trypsin on a monolithic chip device

Monoliths were prepared inside a chip using the recipe described above. Then, the reaction cascade outlined in Scheme 1 was applied. The reaction cascade entailed (i) the flushing of the Y-part with a 5 wt % solution of **1** in ethanol. The ends of the Y-part were closed with septa, the chip was exposed to the electron beam applying a 22 kGy dose, and then flushed with ethanol. Then, (ii), the chip was flushed with dry $CHCl_3$. Then, the Y-part of the chip was flushed with a solution of the 1st-generation Grubbs catalyst (4 mg/mL in $CHCl_3$). The ends of the Y-part were closed with septa, and the chip was stored at room temperature for 60 min. Next, (iii), the chip was flushed with dry $CHCl_3$ for 30 min and then the Y-part was filled with a solution of 7-oxabicyclo[2.2.1]-hept-5-ene-2-carboxylic acid *N*-hydroxysuccinimidester (**3**) (10 mg/mL in $CHCl_3$). The device was closed with the aid of septa, stored at room temperature for 30 min, and then flushed with dry $CHCl_3$ for 30 min. For the removal of the catalyst, the Y-part was flushed with EVE (10 mg/mL in $CHCl_3$, 100 μL in total), then with dry $CHCl_3$ for 30 min. Finally, the device was dried *in vacuo* at 40°C overnight. Then, the Y-part of the device was filled with a solution of trypsin (6 mg/mL) in 50 m*M* HEPES buffer pH 8.0 After 1 h, excess of trypsin was removed by flushing with water. To further enhance the amount of trypsin bound to the support, the free primary and secondary amino groups of the protein were first reacted with glutaric dialdehyde (GA) by passing a 2.5 wt % solution of GA in water over the column, followed by a 6 mg/mL solution of trypsin in 50 m*M* HEPES buffer pH 8.0.[30] The reaction was allowed to proceed for 1 h at room temperature, then the Y-piece was flushed with water for 1 h. For all flushing steps, flow rates of 5 μL/min via port 2 and 2 μL/min via port 1 were applied. By this approach, several trypsin molecules are grafted onto trypsin, thereby enhancing the total amount of trypsin on the monolithic device. A summary of the entire procedure is given in Scheme 2.

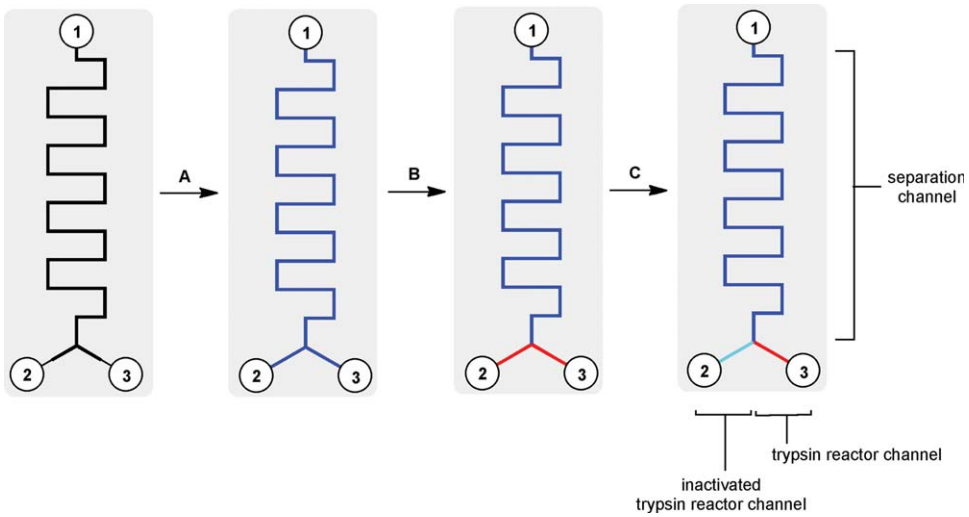
Hydrolysis of Bz-Arg-OEt on a monolithic chip

The trypsin activity was tested via hydrolysis of Bz-Arg-OEt (2 m*M* in 50 m*M* HEPES buffer pH 8.0). For separation of the analytes, the mobile phase was introduced via port 2. (A (95% water, 5% acetonitrile, 0.1% TFA v/v); B (95% acetonitrile, 5% water, 0.1% TFA v/v), isocratic 50% A, 50% B, flow rate: 3 μL/min, UV (254 nm), 25°C). 1 μL of BAEE-solution was injected via channel 3 and flushed with water applying a flow rate of 3 μL/min and 1 μL/min, respectively. The eluate was collected in 100 μL stop solution (50% aqueous methanol solution containing 1% of TFA v/v), and its composition was analyzed by RP-HPLC (0′ 4% B, 15′ 30% B, 17′ 4% B, 30′ 4% B; flow rate = 4 μL/min, UV (254 nm), 25°C, column: Acclaim PepMap 100 (C18, 3 μm, 100 Å, 150 × 0.3 mm I. D., LC Packings, Dionex).

RESULTS AND DISCUSSION

Synthesis and spatially resolved functionalization of polymeric monoliths

Monolithic capillary columns were prepared via electron beam (EB) irradiation-triggered free radical



Scheme 2 Reaction cascade for the spatially resolved immobilization of trypsin on a monolithic chip. Modification sequence: A: I/O: 1 acrylic monomers in, 2 out, 3 out; EB-triggered free radical polymerization; B: I/O: 1 organic solvent in, 2 out, 3 monomer **1** in; EB-triggered free radical polymerization; 1 organic solvent in, 2 out, 3 Grubb's catalyst in; 1 organic solvent in, 2 out, 3 monomer **3** in; 1 buffer in, 2 out, 3 trypsin in buffer in. Analytical configuration: C: 1 detector out (UV), 2 HPLC gradient in (ACN/H_2O mixture), 3 sample/H_2O in. All steps include washing procedures with an appropriate solvent. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

polymerization from a mixture of ethyl methacrylate and trimethylolpropane triacrylate in 2-propanol, 1-dodecanol, and toluene as described previously.[22-25,31] After formation of the monolithic rod, surface functionalization was accomplished via two different routes and visualized by the use of a fluorescent monomer, i.e., by bicyclo[2.2.1]hept-5-en-2-ylmethyl pyrene-1-carboxylate (**2**, Fig. 1).

The first approach entailed the spatially resolved immobilization of bicyclo[2.2.1]hept-5-ene-2-ylmethyl acrylate (**1**) either by using UV in the presence of a photoinitiator (Lucirin TPO-L) or by EB-triggered free radical surface grafting (Scheme 1). For this purpose, parts of the column were covered with Al-foil or Pb and graphite, respectively. The thus prepared spatially resolved functionalized monoliths were used for the immobilization of the 2nd-generation Grubbs initiator $RuCl_2(PCy_3)(IMesH_2)(CHPh)$ (**4**) ($IMesH_2$ = 1,3-dimesitylimidazolin-2-ylidene, PCy_3 = tricyclohexylphosphine). Finally, the monolith with the immobilized catalyst was filled with the fluorescent monomer (**2**) and the successful spatially resolved immobilization of poly-**2** was proofed by fluorescence measurements [Fig. 2(A)]. The alternative, second approach entailed the immobilization of **1** by free radical polymerization-based surface grafting on to the

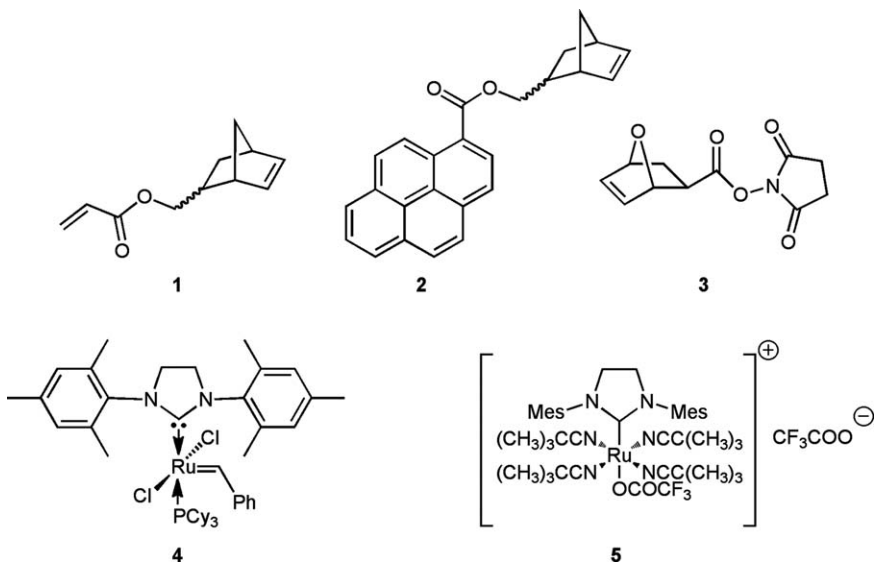


Figure 1 Structure of monomers **1**–**3**, the 2nd-generation Grubbs initiator (**4**), and the photoactive Ru-precatalyst **5**.

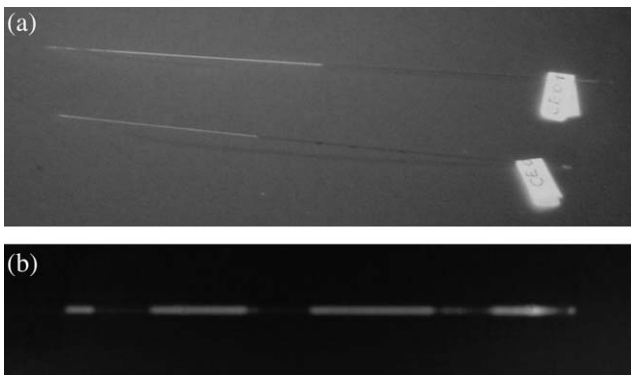


Figure 2 Spatially resolved functionalization of monoliths with poly-**2** by route 1 (A) and by route 2 (B) with reference to Scheme 1.

entire monolith followed by the spatially resolved polymerization of **2** using a UV-triggerable Ru-based precatalyst, i.e., $[Ru(IMesH_2)(CF_3COO)(t\text{-}BuCN)_4^+ CF_3COO^-]$ (**5**) (Scheme 1). For that purposes, a mixture of **2** and **5** was filled into the monolithic column and parts of the column were covered with Al-foil. After UV exposure, unbound monomers and polymers were removed by extensive washing. Again, poly-**2** was immobilized onto the monolith in a spatially resolved way [Fig. 2(B)]. No leaching of poly-**2** after extensive flushing of the capillary monolith with $CHCl_3$ over a period of several days was observed, which is a good indication for a covalent and thus permanent surface grafting.

Next, we filled a chip device with a monolithic polymeric matrix and checked for the separation capability of such a device. Chip devices, which enable for managing the flow through various inlets and outlets and which consist of bonded borosilicate glass with a powder blasted channel structure and which can resist pressures up to 100 bar were used. Advantageously, a monolithic stationary phase, which is known for a low backpressure, effectively prevents the chip from damage and was prepared inside the chip applying EB-triggered free radical polymerization. The monolithic structure that formed inside the chip device is shown in Figure 3. Figure 4 displays a chromatogram of the on-chip separation of different proteins (ribonuclease A, insulin, cytochrome c, lysozyme, albumin), which were injected via port 2 and allowed to exit via port 1. There, good resolution of the signals with excellent peak widths at half height ($3.4<\omega_{0.5}<5.5$ s, Supporting Information) is in a suitable range demonstrating the potential of such devices.

Covalent immobilization of trypsin on a monolithic device and determination of the trypsin activity

Next we applied the newly developed chemistry to the spatially resolved immobilization of proteins on monoliths. For that purposes, an active ester, i.e., 7-oxabicyclo[2.2.1]hept-5-ene-2-carboxylic acid *N*-hydroxysuccinimide ester (**3**) was used as a graft monomer. From the above-described approaches, the one that entailed the spatially resolved EB-based functionalization of the monolith with **1** followed by the ROMP-based functionalization of the poly-**1**-derivatized monolith was chosen for the further process. Briefly, a monolithic column was prepared inside a chip by introducing the reactants via port 1. Then, a solution of **1** was injected via port 3 and allowed to exit via 2 (Scheme 2). After filling, monomer **1** was surface-grafted applying EB curing. Then a solution of $RuCl_2(PCy_3)_2(CHPh)$ was introduced via port 3 and allowed to exit via port 2. After careful washing, a solution of **2** was introduced via port 3 and allowed to exit via port 2. After surface grafting was complete, the catalyst was removed via addition of EVE, then a solution of trypsin was pumped through the Y-part of the device (inlet via port 3 and exit via port 2). Trypsin was chosen as a model protein because in dependence of the reaction conditions it may act as a hydrolase or synthase of peptide bonds and for both processes suitable substrates are available. Although the specificity of trypsin is rather broad, it is the most common enzyme

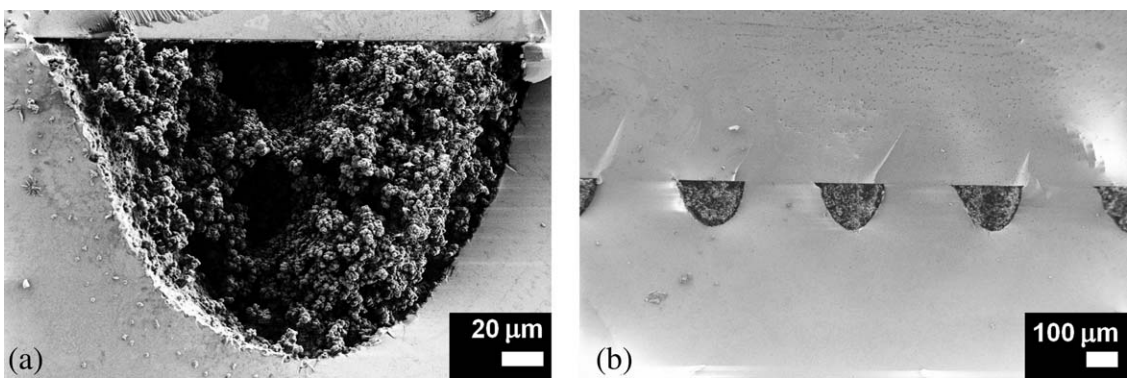


Figure 3 Monolithic structure formed inside the chip.

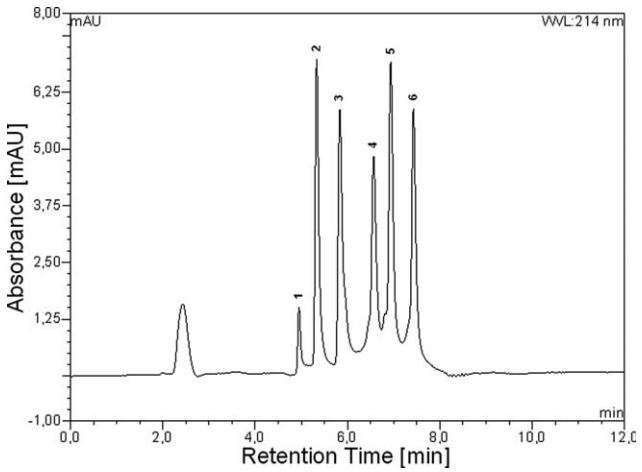


Figure 4 Separation of proteins on a chip-integrated monolith. 1 = additional lysozyme fragment, 2 = ribonuclease A, 3 = insulin, 4 = cytochrome C, 5 = lysozyme, 6 = albumin. Separation conditions: (A) 95% water, 5% acetonitrile, 0.1% TFA; (B) 5% water, 95% acetonitrile, 0.1% TFA; flow 3μL/min, 25°C; UV (214 nm). Gradient: 0–22% (B) within 30 s, 22% 45 s, 22–30% (B) within 45 s, 30–100% (B) within 60 s.

currently used in proteomics and has been immobilized on to various monolithic supports for that degradation purposes.

For on-chip digestion, the channel part containing the immobilized enzyme was kept under a permanent flow of aqueous buffer via port 3 to prevent any inactivation of the protein by organic solvents. For the separation of the digested fragments on the separation channel, an ACN/water gradient was applied via port 2. To prove the principle applicability of this approach, in particular the activity of the immobilized trypsin on the rather short section of the microchannel, Bz-Arg-OEt was digested on the chip using two different flow rates (4 and 6 μL/min, respectively), which influence the dwell period of the substrate on the trypsin channel part. Depending on the flow rate, the complete reaction mixture elutes after 4–8 min, however, without any significant separation of the educt and the hydrolysis product (Supporting Information Fig. 1). This is due to the structure of the monolithic phase where the porosity is not really suited for the separation of low molecular weight compounds. Therefore, the eluted fractions were collected in a stop solution and analyzed offline by μ-HPLC (Fig. 5). Depending on the flow-rate, educt and product signals were found. As expected, the turnover of Bz-Arg-OEt to Bz-Arg-OH was much higher at low flow rates (88% @ 4 μL/min versus 49% @ 6 μL/min).

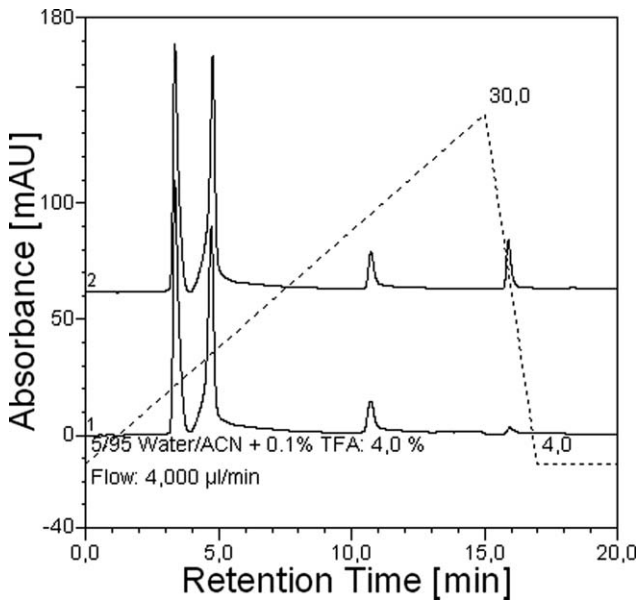


Figure 5 Offline chromatograms of the trypsin-catalyzed, on-chip hydrolysis of Bz-Arg-OEt. t_r: Bz-Arg-OH = 10.5 min, Bz-Arg-OEt = 16 min. Bottom: 6 μL/min, top: 4 μL/min. For the separation conditions refer to the Experimental.

CONCLUSIONS

In summary, we have developed a ROMP-based approach for the spatially resolved immobilization of functional monomers and enzymes on monolithic devices and successfully applied the basic methodology for the spatially resolved immobilization of trypsin on a polymeric monolithic chip device. Thereby, a new approach for the spatially resolved functionalization of capillary monoliths by the use of a recently developed UV-triggerable Ru-catalyst was introduced. This work is a step toward integrated devices, which may serve simultaneously as a catalytic support and separation medium. The monolithic supports themselves have to be further improved concerning the prevention of unspecific protein adsorption and the ability to separate molecules in the low mass range. Finally, the described approach combining the EB-mediated monolith synthesis and ROMP-functionalization for the enhancement of the amount of functional surface groups is not limited to microanalytical devices.

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References

1. Svec, F.; Kurganov, A. A. J Chromatogr A 2008, 1184, 281.
2. Švec, F.; Peters, E.; Sykora, D.; Yu, C.; Frechet, J. M. J. J High Resol Chromatogr 2000, 23, 3.
3. Švec, F.;Tennikova, T. B., Eds. Historical Review; Elsevier: Amsterdam, 2003.
4. Švec, F.; Tennikova, T. B.; Deyl, Z. Monolithic Materials: Preparation, Properties and Application; Elsevier: Amsterdam, 2003.
5. Švec, F. LC-GC: LC Column Technol Suppl 2004, 18.

6. Švec, F.; Huber, C. G. Anal Chem 2006, 78, 2100.
7. Švec, F. J Chromatogr B 2006, 841, 52.
8. Švec, F.; Geiser, L. LCGC North Am 2006, 24 (S4), 22.
9. Švec, F. J Sep Sci 2004, 27, 17.
10. Chuda, K.; Jasik, J.; Carlier, J.; Tabourier, P.; Druon, C.; Coqueret, X. Radiat Phys Chem 2006, 75, 26.
11. Mair, D. A.; Schwei, T. R.; Dinio, T. S.; Svec, F.; Fréchet, J. M. J. Lab Chip 2009, 9, 877.
12. Stachowiak, T. B.; Švec, F.; Frechet, J. M. J. J Chromatogr A 2004, 1044, 97.
13. Sun, X.; Yang, W.; Pan, T.; Woolley, A. T. Anal Chem 2008, 80, 5126.
14. Tan, A.; Benetton, S.; Henion, J. D. Anal Chem 2003, 75, 5504.
15. Wirth, M. J. Anal Chem 2007, 79, 800.
16. Yu, C.; Švec, F.; Fréchet, J. M. J. Electrophoresis 2000, 21, 120.
17. Yu, C.; Davey, M. H.; Švec, F.; Fréchet, J. M. J. Anal Chem 2001, 73, 5088.
18. Yu, C.; Xu, M.; Švec, F.; Fréchet, J. M. J. J Polym Sci Part A: Polym Chem 2002, 40, 755.
19. Buchmeiser, M. R. Polymer 2007, 48, 2187.
20. Buchmeiser, M. R. J Chromatogr A 2004, 1060, 43.
21. Buchmeiser, M. R. J Sep Sci 2008, 31, 1907.
22. Bandari, R.; Knolle, W.; Prager-Duschke, A.; Buchmeiser, M. R. Macromol Chem Phys 2007, 208, 1428.
23. Bandari, R.; Knolle, W.; Buchmeiser, M. R. Macromol Symp 2007, 254, 87.
24. Bandari, R.; Elsner, C.; Knolle, W.; Kühnel, C.; Decker, U.; Buchmeiser, M. R. J Sep Sci 2007, 30, 2821.
25. Bandari, R.; Knolle, W.; Buchmeiser, M. R. J Chromatogr A 2008, 1191, 268.
26. Beier, M. J.; Knolle, W.; Prager-Duschke, A.; Buchmeiser, M. R. Macromol Rapid Commun 2008, 29, 904.
27. Schlemmer, B.; Bandari, R.; Rosenkranz, L.; Buchmeiser, M. R. J Chromatogr A 2009, 1216, 2664.
28. Löber, A.; Verch, A.; Schlemmer, B.; Höfer, S.; Frerich, B.; Buchmeiser, M. R. Angew Chem Int Ed 2005/2008, 120, 9279.
29. Wang, D.; Wurst, K.; Knolle, W.; Decker, U.; Prager, L.; Naumov, S.; Buchmeiser, M. R. Angew Chem Int Ed 2005/2008, 120, 3311.
30. Boulares-Pender, A.; Prager-Duschke, A.; Elsner, C.; Buchmeiser, M. R. J Appl Polym Sci 2009, 112, 2701.
31. Bandari, R.; Knolle, W.; Buchmeiser, M. R. Macromol Rapid Commun 2007, 28, 2090.