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Micro- and Nanopatterned Star Poly(ethylene glycol) (PEG) Materials Prepared by UV-Based Imprint Lithography

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A UV-based imprint lithography method is used for the direct surface structuring of hydrogel-based biomaterials, which are prepared from a family of tailor-made star poly(ethylene glycol) formulations. Bulk star poly(ethylene glycol) (PEG) hydrogels are fabricated by cross-linking acrylate-functionalized star PEG macromolecules. Cross-linking is achieved by radical reactions initiated by UV irradiation. This UV-curable star PEG formulation allows templating of mold structures to yield a stable, stand-alone, elastomeric replica of the mold. In particular, when a secondary, soft mold is used that consists of a perfluorinated elastomer with inherent excellent release properties, nanometer-sized features (down to 100 nm) can be imprinted without specialized equipment. The applied UV-based imprint lithography is a fast and simple technique to employ for the direct topographic structuring of bulk PEG-based biomaterials. The UV-based imprinting into the star PEG prepolymer by means of a perfluorinated, soft mold can be carried out on the bench top, while nanoscale resolution is demonstrated.

Introduction

For bioanalytical devices such as biosensors and in biomedical research such as tissue engineering, the detailed structure and physical properties of the surfaces in contact with proteins and living cells are of critical importance in terms of protein adsorption and cellular responses. To control and take advantage of specific and directed protein interaction and cell adhesion, the unspecific binding processes should be suppressed. Correspondingly, several biocompatible and nonadhesive coating materials have been developed, and the methodologies to prepare such coatings have been optimized.[1−7] The most widely used material for this purpose is based on poly(ethylene oxide) (PEO), also called poly(ethylene glycol) (PEG).[8] This polymer is biocompatible and non-cytotoxic, and it meets the requirements to eliminate undesired, unspecific interactions with biological (macro)molecules.[1] To enable specific and directed interaction with cells, usually bioactive molecules, for example, peptides, proteins, or sugars, are tethered to such surfaces so that they can interact specifically with their binding partners.[9,10] This so-called cell patterning is routinely achieved by soft lithography techniques, such as microcontact printing.[11,12]

Interestingly, several studies have revealed that not only the chemistry of the substrate but also the topography may influence cell adhesion.[12−16] Decades ago, it was found that fibroblasts adopted their shape and aligned to topographic structures (i.e., micrometer-sized grooves), a process denoted "contact guidance".[17,18] More recent investigations have confirmed the alignment of other cells on topographically patterned surfaces with feature sizes comparable to the dimensions of the cells.[16,19−21] Hence, cells and viruses were confined in, for instance, micrometer-sized wells.[22,23] Other studies demonstrated that protein adsorption and cell adhesion were increased on a nanostructured PEG substrate when compared with bare PEG substrates.[24] This effect was ascribed to the increase in hydrophobicity (due to trapped air) and to the increased surface area. Although it is commonly accepted that cells respond also to nanofeatures, the details have remained scarcely understood to date.[25,26] Hence, it is interesting to study systematically the

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antiadhesive properties of a PEG substrate depending on the geometry and periodicity of an embossed topographic structure, both in the micro- and nanometer range. Furthermore, the topology dependent protein adsorption and the controlled introduction of specific binding sites for cell adhesive proteins may also provide new means to control the "grip" and, consequently, the proliferation of living cells.

Previously, we have reported on the preparation of (ultrathin) coatings of PEG-based star-shaped polymers (star PEGs).[27,28] These macromolecules contain six arms consisting of a statistical mixture of ethylene oxide and propylene oxide in a ratio of 4:1, which can be equipped with a number of functional end-groups. A library of different star PEGs has been synthesized, varying in average molecular weight (i.e., 3000, 12 000, or 18 000 g/mol) and in chemical functionalities, for example, bearing hydroxyl, isocyanate, or acrylate end-groups. Coatings of these macromers on surfaces constitute an inert background ready for further biofunctionalization. For example, isocyanate-terminated star PEGs can react *in situ* with amine functions of (bio)molecules (e.g., proteins or low molecular weight ligands). This reactivity allows postfilm modification, for example, by printing a reactive ink on the star PEG films by means of microcontact printing (μCP).[29]

To create topographic patterns, that is, a relief, on PEG surfaces, it is desired to have bulk material rather than thin coatings. Star PEG gels can be prepared by end-linking of precursor star PEG molecules, such as amino-, isocyanate-, or acrylate-terminated star PEGs (Acr-star PEGs). Acr-star PEGs can be cross-linked by Michael-type additions to nucleophiles, such as bi- or multifunctional amines, thiols, and alcohols.[30−32] Alternatively, they can form networks through radical polymerization upon illumination in the presence of a photoinitiator and a cross-linking compound, which is the approach we use.

Recently, a number of examples of surface structured hydrogels have been reported, in which the topographic patterns were achieved by replica molding, and feature sizes (grooves) as small as 2 μm were reported.[33] However, the hydrogels in these studies were cured thermally or by wet chemistry, which are slow processes and typically take many hours to complete. Polymerization by means of photocuring is preferred because it is fast and simple, yields few side products, and results in a homogeneous network. Moreover, precursor molecules bearing reactive groups that are insensitive to the photoinduced radical reactions may be used, which facilitates postcuring (surface) reactivity.[34] We present here the results on bulk star PEG material, whose surface properties can be directly related to those of the thin yet dense coatings as previously and currently investigated. The results reported concern densely cross-linked networks from Acr-star PEGs, which are cross-linked with triacrylate cross-linker molecules, and which are mechanically stable and elastic materials that only slightly swell in water.

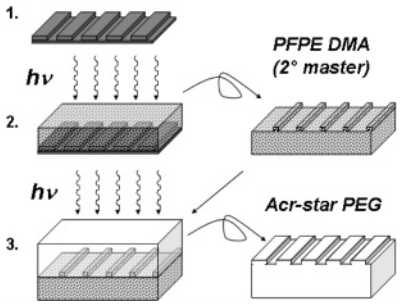

Figure 1. Schematic representation of the three-step process to structure bulk star PEG material. First, a primary (hard) master is fabricated (1), for instance, by photo- or e-beam lithography. This primary master is replicated by the PFPE DMA material by means of UV-curing of the prepolymer against the mold to result in an elastic, secondary master (2). This replica is peeled off and then used as a mold to imprint the structure into the UV-curable star PEG material (3).

Studies on a UV-curable PEG material have revealed that PEG nanostructures can be fabricated with an aspect ratio greater than 1 (∼150 nm wide and ∼400 nm high pillars).[35] Although these results demonstrated that it is feasible to fabricate (PEG) nanostructures, in general, the replication accuracy critically depends on the surface of the mold, especially when nanometer-sized features are concerned. The limitations of nanoimprinting do not lie in the theoretical limit of the features that can be written on the mold, but rather they lie in the practical problems such as adhesion of the different components.[36−38] Notably, too strong adhesion to the mold leads to mold release problems and to damage of the templated structures. In nanoimprint lithography (NIL), these problems are dealt with by treating the mold with release agents, for example, fluorinated coatings, to decrease the sticking of the organic material to the mold. Such mold treatments are required in the case of silicon (primary) masters and in the case when, for instance, poly(dimethyl siloxane) (PDMS) is used as a (secondary) mold.[39] To circumvent these obstacles and also to enable the replication of ultrafine features, we use a perfluorinated polyether (PFPE) material for the fabrication of secondary molds.[40] The elasticity and the low surface energy of this material ensure excellent release, in the first step from the primary (hard) master and the second step from the organic material (i.e., star PEG). Because the PFPE material is elastomeric, it allows conformal contact with the UV-curable star PEG supported by a rigid substrate, and it is easily peeled off mechanically.

Besides the requirement that the soft molds must not be adhesive to the star PEGs, they must also not be swollen by the prepolymers or other components of the precuring mixture, for example, a solvent. Compared to the widely used poly(dimethylsiloxane) elastomer PDMS, a perfluorinated polyether (PFPE) is superior as a mold material to fabricate sufficiently inert, soft 3D templates. This fluorinated polymer has the

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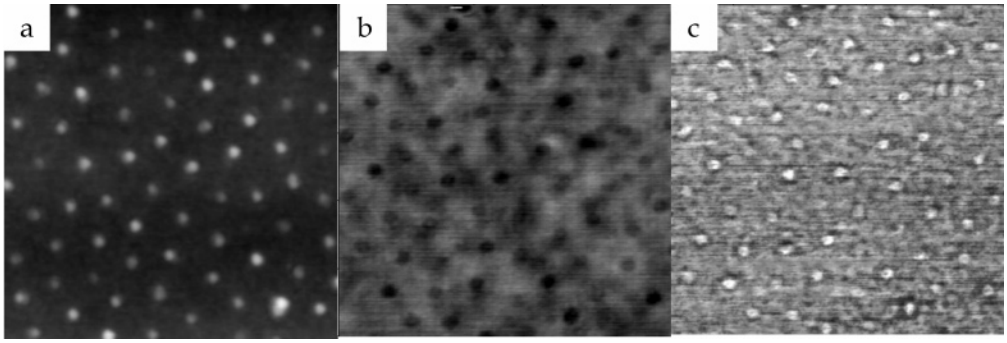

Figure 2. AFM images ($1 \times 1\ \mu m^2$) of (a) a master consisting of silicon decorated with gold dots and (b and c) a PFPE DMA replica thereof: (b) height image and (c) phase image.

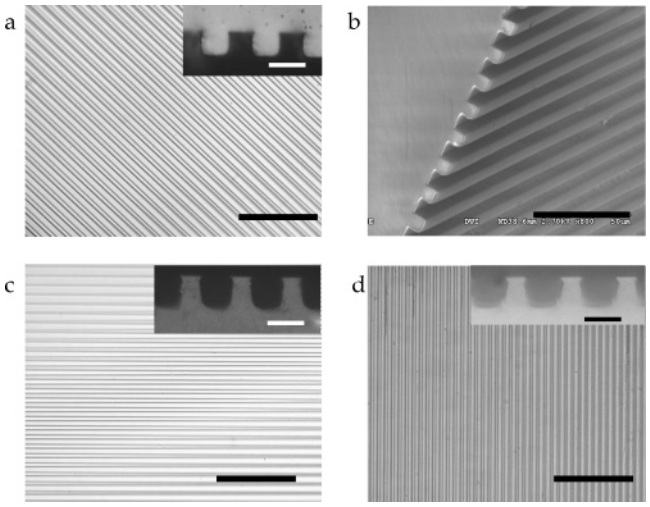

Figure 3. (a, c, and d) Optical micrographs of replicas formed by Acr-star PEG using as the UV-imprinting mold the original silicon master, a PFPE DMA secondary master, and a PDMS secondary master, respectively. Scale bar represents 100 μm. Insets (scale bar represents 10 μm): optical micrographs of cross sections of the bulk Acr-star PEG replicas. (b) Scanning electron micrograph of a cross section of the replica as shown in (a). Scale bar represents 50 μm.

advantageous characteristics of having a very low surface energy and antiadhesiveness, combined with a rather general incompatibility and chemical inertness.[40] Further properties of this material include its high elasticity, transparency, permeability for gases, and negligible swelling in (organic) solvents. Finally, the transparency allows UV-curing by illumination through the mold, which may also be useful for the application.

Materials and Methods

All chemicals were purchased from Aldrich and used as received unless stated otherwise. Solvents were at least analytical grade quality. The silicon masters used in these studies were purchased from Amo GmbH (Aachen).

The synthesis of the acrylate-functionalized star PEG variant was performed according to the following procedure. Prior to the end-capping reaction, the hydroxyl-terminated star PEG (Dow Chemicals) was dried thoroughly for 4 h at 10^{-2} mbar at 80 °C oil bath temperature. Under a nitrogen atmosphere, 0.4 g (1.3 equiv) of acrylic anhydride was slowly added to a mixture of 5.0 g of the star PEG prepolymer and 0.3 mL (1.5 equiv) of water-free pyridine in 15 mL of toluene. The resulting mixture was stirred for 12 h at room temperature. After removal of the solvent and pyridine, the crude product was dried at room temperature for 8 h at 10^{-2} mbar. Unreacted acrylic anhydride was separated from the product by washing with dry ether three times, leaving the product as a colorless oil. The purified product was dried at room temperature for 8 h at 10^{-2} mbar. The purity of the product was verified by NMR. ^{1}H NMR ($CDCl_3$; 300 MHz) δ/ppm: 1.12 (s, 3H, -CH_3 PEG), 3.4−3.8 (m, PEG), 4.24−4.48 (m, 2H, R−C$\underline{H_2}$−O−CO−C_2H_3), 5.80−5.88 (m, 1H, R−O−CO−CH=C$\underline{H_2}$, *cis*), 6.05−6.20 (m, 1H, R−O−CO−C$\underline{H}$=CH_2), 6.36−6.48 (m, 1H, R−O−CO−CH=C$\underline{H_2}$, *trans*). THF based GPC was carried out to rule out cross-linking; a single peak with $M_w/M_n = 1.069$ was found. IR (KBr): $\nu = 3578$ (w), 3514 (w), 2867 [s, ν (CH2, CH3)], 2239 (vw), 2139 (vw), 1961, 1724 [m, ν (-CO)], 1635 [w, ν (−C=C)−], 1580 (vw), 1455 (s), 1348 (s), 1295 (s), 1250 (s), 1108 [vs, ν (−C−O−C−)], 1042 (s), 995 [s, δ (R−CH=CH_2)$_{oop}$], 950 [s, δ (R−CH=CH_2)$_{oop}$], 880 (s), 847 (s), 812 (m), 663 (w), 523 (m).

The functionalization of PFPE diol (Solvay Solexis) with acrylate end-groups was performed according to a procedure adopted from the literature.[40,48] Typically, 0.86 mL (6 mmol) of 2-isocyanatoethyl methacrylate and 50 μL of dibutyltin diacetate (DBTDA) as a catalyst were added to a solution of 5.72 g (3 mmol) of PFPE diol ($M_n \sim$ 1900 g/mol) in 2 mL of 1,1,2-trifluoro-1,2,2-trichloroethane (Freon 113). The reaction mixture was stirred for 24 h at 50 °C, after which the solvent was removed. The crude product was purified by multiple precipitations in petrol ether or hexane. The purity of the product (PFPE DMA) was verified by NMR. ^{1}H NMR (10% $CDCl_3$ in Freon; 300 MHz) δ/ppm: 6.18 (s, 2H, *cis*-H), 5.62 (s, 2H, *trans*-H), 5.35 (b, 2H, NH), 4.50 (m, 4H, CH_2 PFPE), 4.31 (t, 4H, C$\underline{H_2}$−O), 3.58 (m, 4H, C$\underline{H_2}$−N), 2.13 (s, 6H, CH_3).

UV-based imprinting of PFPE DMA was performed according to the following procedure. Typically, 1 g of PFPE DMA was dissolved in 0.5 mL of Freon in the presence of 10 mg (1 wt %) of benzoin methyl ether as a photoinitiator. After homogenization, the solvent was evaporated under a mild stream of nitrogen. The viscous prepolymer mixture was drop-casted on a master substrate and was degassed by evacuation in a desiccator (∼4 min) to remove any air bubbles. The evacuated sample was covered with a transparent foil to make a flatter film (which was advantageous for microscopy studies). The sandwich structure was transferred to a glovebox filled with nitrogen, where UV-curing was carried out by illuminating the sample for typically 20 min under a UV lamp (λ = 366 nm) positioned ∼8 cm above the sample. After curing, the foil was removed from the backside and the elastomeric replica was mechanically peeled off.

UV-based imprinting of acrylate star PEG was carried out in analogy to the procedure described for PFPE DMA. In this case, acetone was used as a solvent for the star PEG precursor. Typically,

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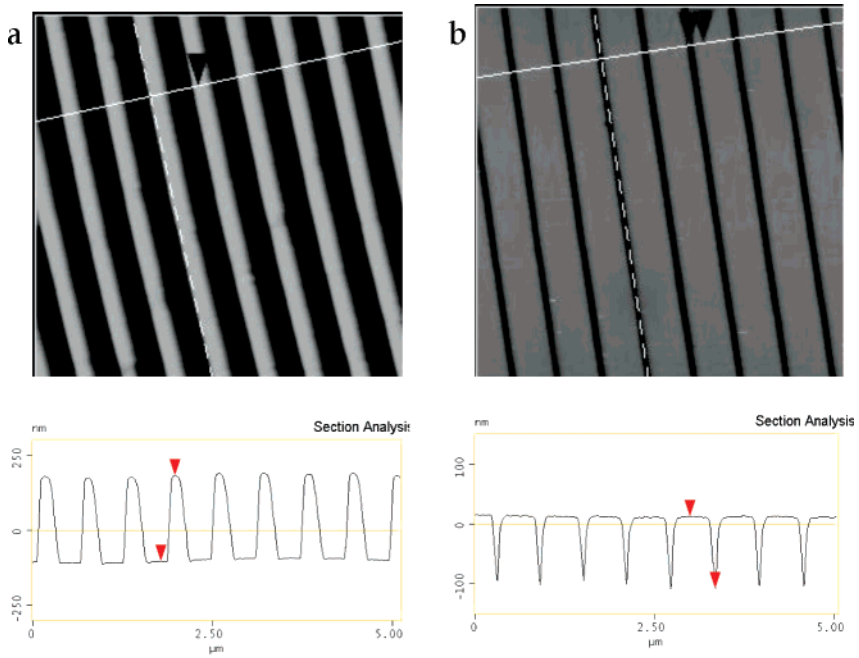

Figure 4. Atomic force micrographs (5×5 μm^2) of replicas formed by (a) PFPE DMA and (b) Acr-star PEG using the replica from (a) as the secondary master. Shown are lines of 100 nm in width (protrusions in (a) and depressions in (b)) spaced by 500 nm.

200 μL of the star PEG was mixed with an acetone solution of the photoinitiator (1 wt %) and pentaerythritol triacrylate (PETA) as a cross-linking agent (5 wt %). After thorough mixing and subsequent evaporation of the solvent, the prepolymer mixture was drop-casted on the (secondary) master substrate, the sample was degassed, and UV-curing was carried out on the bench top, under ambient conditions (for 20 min). The star PEG bulk replica was peeled off mechanically in the case of sufficiently thick and robust bulk replicas. In the case of thinner and more fragile films, the release of the star PEG replica from the PFPE DMA mold was aided by adding a droplet of distilled water at the circumference of the star PEG and letting the water enter the interface. After separation, the star PEG replica was dried under nitrogen flow. Possible, but limited, swelling during a short time did not disturb the pattern.

Scanning force microscopy (SFM) was performed using a Digital Instruments Multimode probe equipped with a Nanoscope IIIa controller (Veeco Instruments, Santa Barbara, CA) and a Molecular Imaging PicoSPM scanning force microscope. Imaging was done in the tapping and acoustic modes using standard silicon cantilevers ($k \approx 2$ N/m, $f_0 \approx 70$ kHz; Nanoworld, Neuchâtel, Switzerland). Images were edited with Nanoscope software (v5.12r5 Digital Instruments, Veeco, Santa Barbara, CA) and Femtoscan software (Advanced Technologies Center, Russia).

The scanning electron microscopy image in Figure 3b was taken with a S-3000N (Hitachi) microscope using an accelerating voltage of 2.7 kV and a working distance of 39 mm, and the one in Figure 5c was taken with a S4800 (Hitachi) microscope (accelerating voltage $= 1.0$ kV and working distance $= 8$ mm).

Light microscopy was performed on an Axioplan 2 Imaging microscope (Carl Zeiss AG), that is, an optical reflection microscope equipped with a differential interference contrast (DIC) module. Pictures were taken using an AxioCam MRc digital camera and analyzed using the AxioVison 3.1 software package (Carl Zeiss AG).

Results and Discussion

The imprinting into the star PEG is performed in a three-step process (Figure 1). In the first step, a master structure is prepared from, for example, silicon, quartz, or glass by established patterning techniques, such as photolithography and electron-beam lithography (EBL), followed by pattern transfer into the substrate by an etching process.[41,42] The choice of the lithographic technique is based on the desired feature size (down to nanometer resolution), the area of the patterned surface, and the geometry of the features. In the second step, the (hard) primary master is replicated by a soft polymer to yield an elastomeric replica, which is used as a mold in the third step to imprint the structure into the Acr-star PEG.

The UV-curable prepolymer, which is used to prepare the secondary mold, is a perfluorinated polyether (PFPE) derivative ($M_w \sim 1900$ g/mol) with methacrylate end-groups, which enable the use of UV-based imprinting as the technique of choice for mold preparation. The perfluorinated polyether dimethacrylate (PFPE DMA)[40] replica is peeled off as a self-supportive elastomeric film, which is stable, transparent, and flexible. As shown previously by De Simone et al.[43] (who used a prepolymer with a molecular weight of 3800 g/mol), a nanostructured silicon master could be replicated with superior conformity compared to PDMS. The accuracy of replication by PFPE DMA can go even further down an order of magnitude, as was recently reported[44] and is also demonstrated here. Figure 2 shows the replication of a gold nanodot decorated silicon surface.

This specific master (Figure 2a) was prepared according to a procedure denoted as block copolymer micelle nanolithography.[45,46] The replication of this substrate resulted in nanometer-sized indents in the peeled-off elastomeric film, which demonstrates the accurate replication of extremely small features (the nanodots are $\sim$5 nm in size while spaced at a mutual distance of $\sim$100 nm). The hexagonal pattern (originating from the self-organization of the block copolymer micelles into a close-packed monolayer) is easily recognized in the atomic force microscopy (AFM) images of the replica, especially from the phase image (Figure 2c). The demonstrated accurate nanoreplication drastically expands the scope of soft lithography methods and holds great promise for applications where PDMS fails due to its poor replication ability in the low-nanometer regime or due to swelling or deformation during the imprinting process.[43,47]

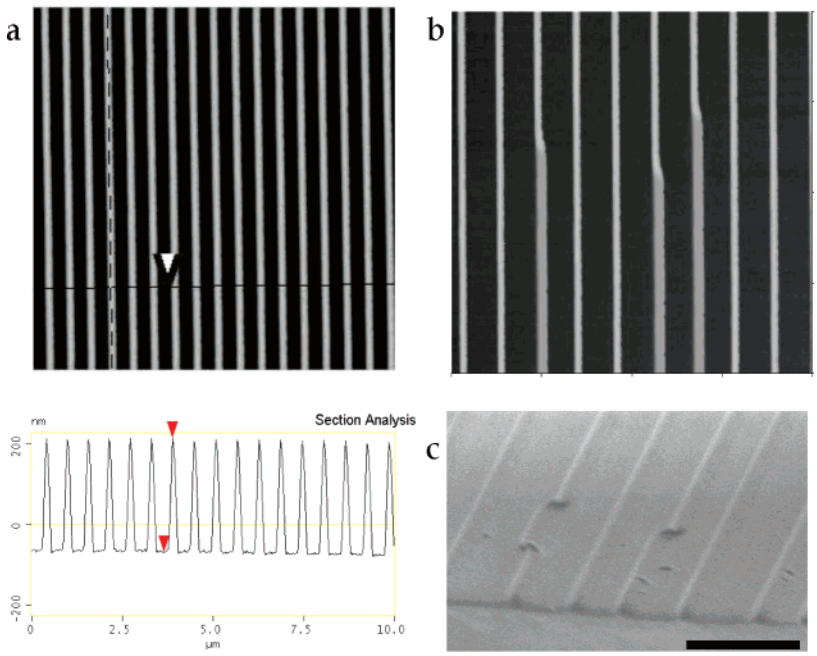

Figure 5. (a) and (b) Atomic force micrographs ($10 \times 10\ \mu m^2$) of an Acr-star PEG replica using the silicon master directly as the primary mold. Shown are 100 nm wide and 282 nm high ridges spaced by 500 nm and 1 μm, respectively. (c) Scanning electron micrograph of the hydrogel replica; scale bar represents 2 μm.

A particular advantage of our star PEG formulations is that they are viscous liquids that do not crystallize at ambient temperature, but they conform without problems to the master relief, which makes the use of a solvent superfluous. As an additional advantage, the polymer nature of the star PEG precursors ($M_w \sim 12000$ g/mol) ensures that the precursor liquid will not penetrate into the elastomeric mold and potentially deform the pattern.

Using this strategy of imprinting to transfer the pattern of a hard master (directly or via a secondary master) into the soft star PEG, stand-alone, bulk hydrogel replicas are prepared. Figure 3 depicts the results for the UV-curable Acr-star PEG. On the micrometer scale, replication is successful using either a secondary master, for example, PDMS or PFPE DMA, or even the primary (silicon) master, resulting in large area, homogeneous bulk star PEGs showing little or no defects. Scanning electron microscopy measurements revealed that the surface of the hydrogel replica is smooth and clean (Figure 3b).

For the replication of nanostructured masters (of quartz and silicon), a PFPE DMA replica was used as the secondary master to imprint into the UV-curable star PEG. Figure 4 depicts the AFM images and corresponding cross sections of the secondary master and of the bulk star PEG replica.

The cross section of the PFPE DMA replica (Figure 4a) shows that the 100 nm wide grooves on the primary silicon master are accurately replicated to result in 100 nm wide "walls". In addition, the height of the protrusions (286 nm) demonstrates the ability of the fluorinated material to form stand-alone 3-D structures with appreciable aspect ratios. The star PEG secondary replica (Figure 4b) shows shallower grooves than expected, while the lateral dimensions are a correct positive copy of the primary master. The apparent shallowness of the grooves is ascribed to the limited ability of the SFM tip to follow the contours of the grooves, that is, convolution with the tip shape.

The same silicon master that was used to prepare the secondary mold from PFPE DMA (Figure 4a) was also used as the primary master to imprint nanometer features *directly* into the hydrogel material. The AFM image demonstrates that a hard, silicon master can be used as the imprinting mold to structure bulk star PEG material, even at the nanoscale level. Also, in this case, the aspect ratio of 3 is reached; the ridges are 282 nm high (and 100 nm wide). Interestingly, however, the hydrogel's mechanical properties are not as robust as those of the PFPE material, and the ridges were observed to collapse occasionally (Figure 5b and c).

Further research will elucidate which aspect ratios are feasible to be reached with the PFPE and star PEG materials and will make clear how the mechanical integrity of the latter can be improved (e.g., by increasing the cross-linking density).

Conclusions

It was demonstrated that novel, UV-curable star PEG precursors can replicate topographically structured masters with a resolution down to 100 nm, resulting in 3-D bulk star PEGs. The (nano-) structuring was performed according to UV-based imprint lithography, taking advantage of a perfluorinated, elastomeric mold, which was replicated from a primary, hard master. It was shown that the perfluorinated material PFPE DMA has excellent replication properties down to a few (5−10) nanometers. On top of that, the elastomeric mold, which was prepared and used as a secondary master, has intrinsic, good mold release properties, which makes it very suitable to use as an imprinting mold to structure the UV-curable star PEG, without the need for mold treatments. This strategy enables imprinting into star PEG materials, which can be carried out on the bench top, with nanoscopic resolution.

These elastomeric bulk star PEGs will be applied as biointerfaces in cell studies. Basically, the PEG nature of the used star PEG precursor material promises antifouling properties of the surface. Nevertheless, topographic patterns may invoke cell binding processes to take place at the globally cell-repellent but locally micro- or nanostructured star PEG substrate. The effect of micro- and nanotopography on this cell-repellent substrate is

currently being investigated in several cell experiments, and the preliminary results indicate that the imprinted star PEG substrates are indeed (still) protein- and cell-repellent.

Further research aims at the development of several interesting combinations of topographically and chemically patterned substrates, and the mutual effect on cell binding, spreading, growth, and proliferation will be studied.

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