Chapter 12
Polymer Nanostructuring by Two-Photon Absorption

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Abstract Two-photon polymerization (2PP) is an innovative technology that in recent years showed a tremendous potential for three-dimensional structuring of photopolymers at the submicron scale. It is based on the nonlinear absorption of ultrashort laser pulses in transparent photosensitive materials. 2PP has been so far exploited in various fields, including photonics, microfluidics, regenerative medicine and MEMS prototyping. The versatility of this technology relies also on the photomaterials; indeed, polymers are easy to process, low cost and they allow the tailoring of their chemical and mechanical properties. 2PP nanotechnology is here exploited to produce micro and nanostructures that can be easily customized both in the geometry and in polymer functionalization. In particular, atomic force microscopy tips are fabricated on top of commercial cantilevers to demonstrate the technology feasibility and customizability. Moreover nanoporous membranes that can be fabricated by 2PP as a single custom product or as a mould for mass production through replica moulding are realized to evaluate the scalability of the fabrication process.

12.1 Scientific and Industrial Motivations

In recent years, the development of industries capable of supplying increasingly customized products according to the needs and desires of customers is taking hold. The concept of *on-demand fabrication* is therefore attracting much attention in several fields. Laser fabrication is in general a flexible and rapid prototyping process for the creation of a variety of microstructures in a broad range of materials, but it allows only serial fabrication processes. This aspect, which is highly desirable for customization, poses some limitations for mass production and even for mass customization.

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Two-photon polymerization (2PP) by femtosecond laser pulses is a fabrication technology that showed great potential in the fabrication of three-dimensional (3D) polymeric sub-micron structures with resolution down to 100 nm [1–3]. 2PP process is based on a photochemical polymerization reaction triggered by the two photon absorption that occurs in the focal point of a tightly focused femtosecond laser pulse. By moving the focal spot in a UV photopolymerizable transparent photoresist, complex patterns of polymerized material can be realized; the non-irradiated polymer can be removed by a development step that leaves free-standing 3D microstructures of polymerized material. Two-photon polymerization is a highly reconfigurable lithographic technique that allows a high control of the fabrication parameters and shows many advantages such as the submicron resolution and the ability to directly write three-dimensional structures without constraints related to their shape. This technology is thus very promising in the frame of prototyping since it allows an unprecedented freedom in the product customization and provides an important tool to create nanostructures otherwise not feasible. The areas of application are numerous and range from the biomedical sciences to telecommunications, micromechanics and microfluidics [5–12]. The limitations linked to its serial nature should be overcome to promote this fabrication technology from the laboratories to the industries. In some cases this can be done by exploiting the potential of 2PP to fabricate the master mould to be used in replica moulding (REM), i.e. a promising technology for duplicating the shape, size and pattern of features present on a master [13].

The main goal of this work is therefore to tackle the potentialities for an industrialization of the two-photon polymerization process by demonstrating its capability to produce micro and nanostructures that can be easily customized both in their geometry and in material functionalization and the scalability of products whenever replica moulding is coupled to this technology.

2PP is a highly flexible rapid prototyping technology allowing a high control of the fabrication parameters. Photopolymers are low cost, easy to process and tailorable in their chemical and mechanical properties. The combination of these aspects allows satisfying the need for products and services that can be adapted to the customer specific requirements.

The intermediate objectives to successfully achieve the main goal are then the development of photopolymerizable polymeric materials with peculiar chemical properties and of the laser processing procedures that allow the nanostructuring of these materials by 2PP with specific resolution ranges. In particular, a relevant case study has been identified to validate these approaches in the manufacturing of atomic force microscopy (AFM) tips and of nanoporous membranes. Because of the wide diffusion of this technology in many research fields, the customization of the tips both in terms of the geometry of the structure and in terms of their mechanical and chemical properties has potentially high impact for an on-demand product customization, perfectly matching the trend that industry is experiencing in the products personalization.

In the following sections the problem tackled by our research is introduced by evaluating the state of the art of the proposed technology applied on the envisaged applications. Afterwards the achieved results are reported first by showing the choice of the polymer through tests on its polymerizability, and then the fabrication of AFM tips is shown both on glass coverslips and in commercial AFM cantilevers. The fabricated AFM tips have been validated by nanoindentation measurements and the obtained results on biologically relevant soft materials are reported. At last preliminary experiments on nanoporous membrane fabrication by replica moulding are introduced by showing the process exploited to fabricate both the master by 2PP and the membranes by replica moulding.

12.2 State of the Art

Many technologies are available to fabricate microstructures. The leading technology for AFM tips is photolithography, usually on hard materials such as silicon and its compounds. Unfortunately, the mechanical properties of these materials cannot be tailored with the freedom which is typical of polymers; moreover it is very difficult to produce curved features, and they require a complex and expensive multi-step process. Polymers can be processed by photolithography or by electron beam lithography with very high lateral resolution, but only in a single layer fashion, preventing the realization of complex geometries. Stereolithography has the ability of producing more complex structures but with a lower resolution, especially in the vertical direction, and always with a layer-by-layer approach.

An innovative technology for 3D structuring of photopolymers at the sub-microscale able to overcome these limitation is two-photon polymerization (2PP), based on the nonlinear absorption of ultrashort pulses in transparent photosensitive materials [1–4]. Because of the nonlinear nature of the process, a lateral resolution beyond the diffraction limit can be realized by controlling the laser pulse energy deposition. Moreover, the absorption along the beam propagation direction is tightly confined in the focal region. As a result, the technique provides much better structural resolution and quality than the aforementioned techniques. Microstructuring of photosensitive materials by 2PP is effective for the fabrication of 3D structures with a resolution of 100 nm or better, achievable through the combination of computer-controlled positioning systems and, typically, near-infrared laser oscillators.

The potential applications of 2PP technology cover diverse research fields, ranging from the fabrication of photonic elements [5, 6] and of scaffolds for regenerative medicine [7–9], to the prototyping of MEMS [10] and the fabrication of microfluidic components [11, 12]. Another promising application is the fabrication of polymeric tips for Atomic Force Microscopy (AFM). AFM tips are currently manufactured with standard microfabrication technologies, by using hard inorganic materials (such as silicon nitride, silicon, gold) and requiring expensive and time-consuming facilities. The main advantages of polymeric AFM tips realization by 2PP relies on the combination of a cheap manufacturing process and the appealing capacity of customization. AFM tips can be tailored in terms of both geometry and materials; in particular the customization of mechanical and chemical properties may be realized by exploiting polymers with specific functionalities for a targeted application. The first AFM tips structured by 2PP were demonstrated in 2005 by Kim and Muramatsu [14]. Since then only a few related studies were reported and all of them employed the same commercial polymer, which is composed of acrylic and epoxy monomers [15]. The main advantages of acrylic photoresists are their wide commercial availability, the optical transparency, and the ease of processing [16]. Nevertheless, acrylic polymers present poor chemical resistance to organic solvents that hinders the realization of 3D structures to be used in applications requiring chemically resistant elements. Perfluoropolyether polyurethanes (PFPE) are well known macromolecular families that can be used to obtain hydrophobic and chemically resistant materials [17–19]. The synthesis and processing by 2PP of a new PFPE-based have been recently demonstrated by the partners of the project [20]. 2PP fabricated hydrophobic AFM tips were already proposed [14], but the contact angle of the photopolymer was about 70°, too small to be considered a low wettability surface. Adhesion forces between AFM tips and hydrophilic surfaces can be reduced by exploiting hydrophobic self-assembling coatings; anyway these are easily removed by the tip interaction with the surface [14]. The availability of AFM tips directly fabricated with a hydrophobic material, as for tips realized by 2PP structuring of low surface tension polymers, would be advantageous for applications as topographic imaging of biomacromolecules or hydrogels; in fact, the adhesive forces between the standard AFM tips and their hydrophilic surfaces may affect the imaging quality. Moreover, AFM emerged as an essential tool to measure the elastic modulus of soft materials otherwise difficult to be estimated [21]. For example, Cross et al. [22], using the AFM, showed that cancer cells are 70% less stiff than normal cells, but the model used to estimate the elastic modulus approximated the real shape of the AFM tip by a cone or a paraboloid. Reproducing the exact cone or paraboloid shape by 2PP may reduce the errors, which affect this type of measurements.

2PP technology has demonstrated an excellent potential for the fabrication of complex polymeric structures at the microscale, but its serial nature usually hinders a mass production of components. For this reason a parallel replication method of 2PP structures is very desirable.

2D nanoporous membranes have many applications, ranging from optics and electronics, to filtration and purification or biosensing and single-molecule detection. Despite the extensive research carried out in fabrication of nanoporous materials, there are still challenges to create nanoporous systems, mainly related to the poor flexibility in terms of pore shape and uniformity intrinsic to standard lithography and ion-track etching [23]. Replica moulding enables to duplicate the shape, size and pattern of features of a master, usually fabricated on silicon by photolithography, to a polymeric replica by means of an intermediate polymer mould [13]. Productivity, precision and fidelity of REM replication process are largely dependent on the characteristics of polymeric moulding material. To date, poly(dimethylsiloxane) (PDMS) elastomers have been largely used; however, PDMS swells in contact with many solvents and chemicals, and it has some limitations when used to produce very low aspect ratio structures [24]. Photocurable PFPEs have been recently proposed as higher performance moulding materials [25]. In particular, PFPE moulds were recently used to replicate the submicron structures patterned on poly(methyl methacrylate) (PMMA) and polystyrene (PS) substrates by femtosecond laser ablation [26]. 2PP technology would then be a suitable rapid prototyping technology to realize customized masters to be used for nanoporous membranes replica.

12.3 Problem Statement and Proposed Approach

The aims of the work are the validation of the two-photon polymerization technology as a new methodology to fabricate customized micro and nanostructures to be used in various applications and the preliminary demonstration of customized mass production through 2PP and replica moulding technologies. Given the ample range of possible applications for the proposed technology, the attention was focused on the fabrication of atomic force microscopy (AFM) tips, as an archetypical and relevant example of the potentiality of the 2PP technology. AFM and scanning probe microscopy in general are widely diffused technologies exploited in many fields of research for morphological and mechanical material characterization with exceptional spatial accuracy; on the basis of the specific application, different geometries or functionalization of the tip are required. For example, hydrophobic tips are desirable when biological elements are under investigations so as to minimize adhesion forces between the tip and the sample under study; high aspect ratio tips would be beneficial in the analysis of materials with high asperities, while spherical tips would be more appropriate for soft material studies. The second objective, which is of potential interest for the industrialization of the process, was the feasibility study of customized mass production by 2PP. To overcome the limitation of 2PP due to its serial nature REM could be applied to 2PP fabricated master structures. In this way, the high prototyping capability and reconfigurability of 2PP is exploited to realize custom moulds that can be then massively reproduced by means of replica moulding technique. As interesting example for the feasibility of the demonstration, nanoporous membranes have been chosen. Membranes with nanometer-scale features may have several applications, e.g. in electronics, optics, catalysis, selective molecule separation, filtration and purification, biosensing and single-molecule detection.

To achieve these goals, the attention has been first concentrated on the optimization of the 2PP fabrication process for different photoresists, either commercially available or specifically developed. In particular, new photoresists have been developed with tailored chemical and mechanical properties to satisfy the desired hydrophobicity and resistance to solvents. After a preliminary material characterization, attention has been devoted to study and identify the laser processing windows that allow obtaining specific resolution ranges in the fabricated nanostructures for the chosen materials. The obtained results permitted to fabricate prototype tips both on glass coverslip substrates and on commercial cantilevers to be tested for AFM indentation measurements. Soft hydrophilic materials important in biomedical applications such as, for example, regenerative medicine, have been then chosen as test materials to validate the prototypes produced. The attention has been then moved to the choice of the materials to be used both for the realization of the master by 2PP and for the membrane replication by REM. Different conical structures have been fabricated by 2PP as master, which has been used to replicate membranes with different porosities.

The strategy to fulfil the proposed objectives followed a three-step work-plan: (i) development of new functionalized photoresist and definition of the operating parameters of the laser for 2PP fabrication; (ii) demonstration of the realization of hydrophobically functionalized AFM tips by fabricating the tips on commercial cantilevers and by experimentally validating them through AFM nanoindentation measurements; (iii) realization by replica moulding of nanostructured devices such as porous membranes for biosensing applications, whose master has been fabricated by 2PP technology.

12.4 Developed Technologies, Methodologies and Tools

The development of the technology consisted in three steps: first the polymers, offered by industries or synthetized on purpose, were tested to evaluate their photopolymerizability by 2PP. Then the laser writing process was optimized to obtain the desired tip shape. Finally the tips have been fabricated on top of commercial cantilevers to be used in AFM systems.

The achievement of the proposed objectives allowed the development of new materials and the optimization of the fabrication methods for both the proposed case studies, i.e. AFM tips and nanoporous membranes. In fact in order to realize AFM tips, different hydrophobic polymers have been produced and exploited to fabricate microstructures by 2PP. As shown in the following subsections, the optimization of the processes allowed identifying one of these polymers as the best to realize hydrophobic AFM tips in terms of shape and mechanical stability. The chosen polymer has been also exploited in replica moulding process to realize nanoporous membrane with the goal of demonstrating the feasibility of the process.

12.4.1 Realization of AFM Tips

With the aim of obtaining hydrophobic photoresists to fabricate AFM tips with reduced tip-sample interactions, fluoropolymers were chosen among the various classes of polymers. The use of fluoropolymers, in fact, guarantees to achieve the properties of hydrophobicity and solvent-resistance, due to the very low polarizability of C–F bonds. More specifically, perfluoropolyethers (briefly PFPEs) are a class of polymeric oils having the following chain $-(CF_2O)_q(CF_2CF_2O)_p-$ which can be chemically functionalized to obtain photopolymers suitable for standard UV curing processes.

Different PFPE-based photoresist to be effectively structured by 2PP were considered. Their validation in terms of chemical and physical properties is reported in

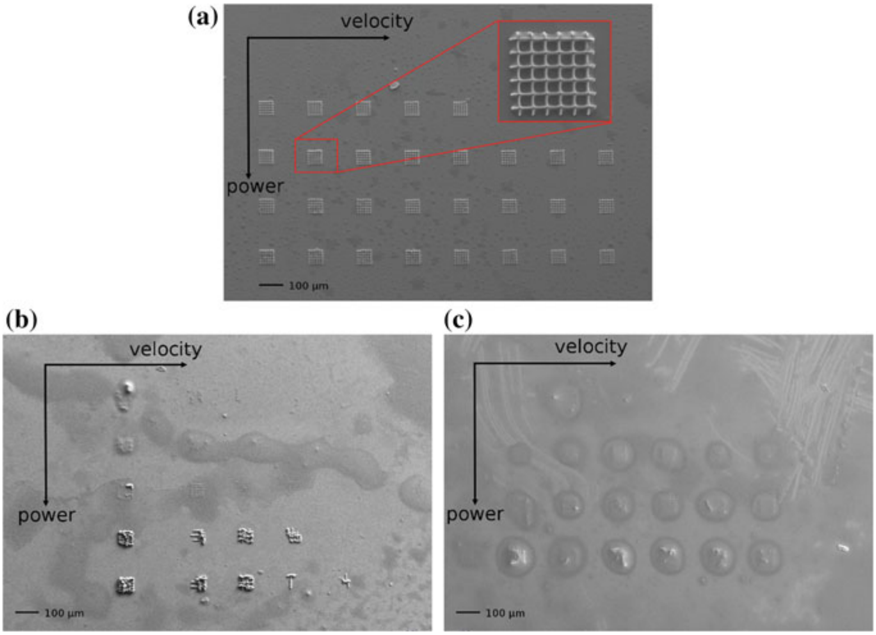

Fig. 12.1 Power-velocity arrays of multilayer grids, power and scanning velocity ranges respectively from 5 mW to 30 mW and from 5 to 250 μm/s. **a** PFPE-PETA, **b** PFPE-TUMA and **c** PFPE-DUTA structures

Sect. 12.5, while here the development of the 2PP technology for these photoresist is described. The testing has been carried out by polymerizing simple structures made by layers of parallel lines, each layer perpendicular to the previous one, and varying laser power and irradiation velocity to identify the optimal processing parameters. The deposited energy is crucial in the 2PP process, since when the energy is not sufficient to generate a critical amount of free radicals in the materials, polymerization will not occur, while if it is too much the material will be damaged, becoming strongly absorbent and spreading the damage to a large surrounding area. These energy values allowed defining a polymerization threshold and a damage threshold for each material.

The laser source employed for 2PP was a Toptica FemtoFiber PRO Er-doped fibre laser, whose second harmonic at 780 nm produces pulses of less than 100 fs at a repetition rate of 80 MHz. Figure 12.1 shows the outcomes of the preliminary tests where the laser power was varied between 5 and 30 mW, while the scan speed between 5 and 250 μm/s. These ranges are shown in the figure along the vertical and the horizontal direction, respectively. The values of laser power and irradiation velocity that gave well-formed lines define an initial fabrication window, included between the polymerization threshold and the damage threshold, from which a finer study of the stability of more complex structures could start.

As visible from Fig. 12.1a, the structures fabricated in PFPE-PETA are generally well formed, which means that with this material a wide fabrication window is available. This is a critical aspect, since it allows a robust fabrication process, where small variations in the laser intensity and in the fabrication environment will not spoil the final result. On the other hand, in PFPE-TUMA and PFPE-DUTA, respectively in Fig. 12.1b, c, the multilayer grids are either non polymerized or damaged, which means that it was not possible to identify a good combination of laser power and fabrication speed for these materials with the employed laser source. The other PFPE based materials tested showed no evidence of polymerization. Given these results, PFPE-PETA has been identified as the only suitable material for 2PP, among the ones considered in this study.

A second aspect that should be taken into account in the development of the optimized fabrication method concerns the geometry of the desired structure. The shape chosen for the AFM tip was a cylindrical base with a hemisphere on top; the sphere, with a well-defined radius of curvature, is necessary for nanoindentation measurements. Indeed it allows a simple modelling of the interaction between the tip and the material, and it is thus required by the mathematical models used to extract the mechanical properties of the sample from the curves acquired with the AFM. The base, instead, prevents any attraction or repulsion between the AFM cantilever and the sample surface. Conical tips have also been proposed for this task, but the smaller curvature radius of the tip was more difficult to measure via scanning electron microscope (SEM) images, and less reproducible, since it varied much with the laser irradiation parameters, so this geometry has been discarded.

To irradiate the volume of such structures with the femtosecond laser, a layer-by-layer irradiation pattern has been chosen, as it is common in many 3D-printing techniques for compact volumes. The tip has been divided in horizontal slices, spaced less than the single polymerized laser line, and each slice was scanned with parallel lines. To study the mechanical stability of these structures, before proceeding with the fabrication on AFM cantilevers, some tips were realized on top of a coverglass with PFPE-PETA. This was necessary to identify the optimal distances between slices and between lines, which give well defined shapes and avoid energy accumulation, due to the excessive superposition of nearby lines. Once completed this first step, stability and repeatability of the tips was studied, by varying laser power and irradiation speed within the previously defined fabrication window, and realizing for each combination nine identical structures.

A parameter scan with power ranging from 10 to 30 mW, and speed from 10 to 100 μm/s is shown in Fig. 12.2. It can be observed how the tips shown in the inset are well formed and reproducible, while the ones with much higher power in the top line are swollen, and the ones in the bottom line, with the lowest power, are collapsed.

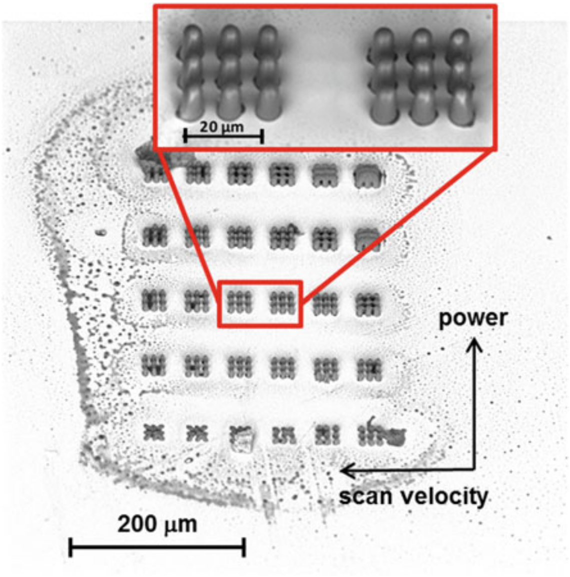

Fig. 12.2 Optimization of the fabrication parameters. Groups of nine identical cylinders with an hemisphere on top have been fabricated for each power-velocity combination. A detail of well-defined structures written at 20 mW, 30 and 50 μm/s is shown in the inset [27]

12.4.2 Realization of Nanoporous Membranes

The realization of nanoporous membranes could be carried out according to two different strategies, i.e. (i) direct fabrication of the membrane by polymerizing the photoresist and (ii) fabrication by 2PP of a master for REM. In the first case the fabrication of the membrane would allow a very high precision in the positioning and shape of the pores, enabling also the formation of very small pores. Since the polymerization process would occur around the pore, large regions should be polymerized and almost no limits due to the resolution of the process would occur. In the second case the fabrication time is shorter, even if the pore dimension is limited to the smallest feature that can be fabricated by 2PP, i.e. by the resolution of the 2PP process.

Taking advantage from the results obtained in the realization of AFM tips, the second strategy has been chosen. The activity has been focused on the fabrication by 2PP on a glass substrate of a stable matrix of cones to be used as master for the membrane replica. The conical tips were made of SZ2080 material, which was proven to be suitable for producing well-defined structures via 2PP. First, the optimal fabrication parameters to achieve cones with very sharp tips have been found, since this would determine the minimum size of the pore achievable after replica moulding and the results are reported in Fig. 12.3a. Secondly, the stability of the fabricated structures has been evaluated; indeed, the cones should have high adhesion to the substrate in order not to detach during the membrane stripping process. No treatment of the glass could be performed; in fact, silanization would increase the photopolymer adhesion, but also the membrane adhesion, hindering the stripping process. Two

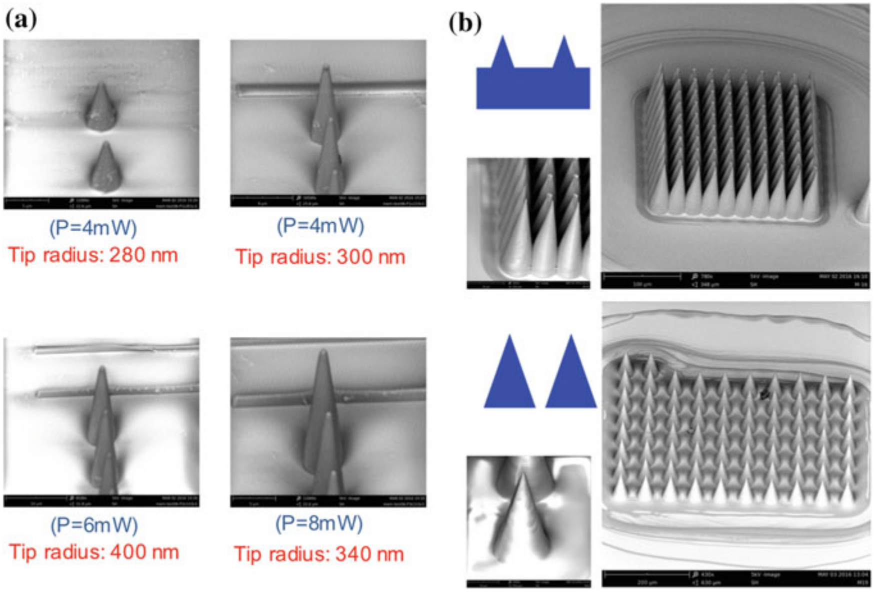

Fig. 12.3 Development of tips matrices to be used as master for nanoporous membranes replica. **a** Optimization of the laser parameters for fabrication of cones with sharp tips. In all the shown cases the distance between irradiated adjacent planes is 0.3 nm and the scan velocity is equal to 0.07 mm/s **b** Optimization of the matrix of cone stability and geometry

different structures of cones with a large basement or larger bases to increase the stability and the adhesion of the cones have been therefore tested. The results reported in Fig. 12.3b show that both strategies allowed obtaining a stable matrix of cones; anyway, cones with large bases and without a basement have been later used since this allowed realizing tips of cones without defects and larger coverslip areas covered by cones.

In order to produce high fidelity membranes as negative replicas of the master, different PFPE-based photoresists have been tested, taking advantage of their low surface tension, which makes de-moulding operations more efficient. Among these, we have decided to employ a UV-curable PFPE-dimethacrylate (PFPE-DMA 4000), synthesized with a reaction between 2-isocyanate ethylmethacrylate and a PFPE macrodiol (average molecular weight of nearly 4000 g/mol). The product of this reaction was cured after adding a photoinitiator and the photo-crosslinked material exhibited a high hydrophobicity with a water contact angle of 113 ± 0.7° and a shear modulus of 0.55 MPa, suitable to have soft membranes that can be easily peeled off from the mould. Replicated membranes were then obtained by means of the following 4-step process: (1) drop-casting of the PFPE photocurable resin on the mould; (2) spin-coating; (3) UV curing; and (4) de-moulding of crosslinked nano-structured layers.

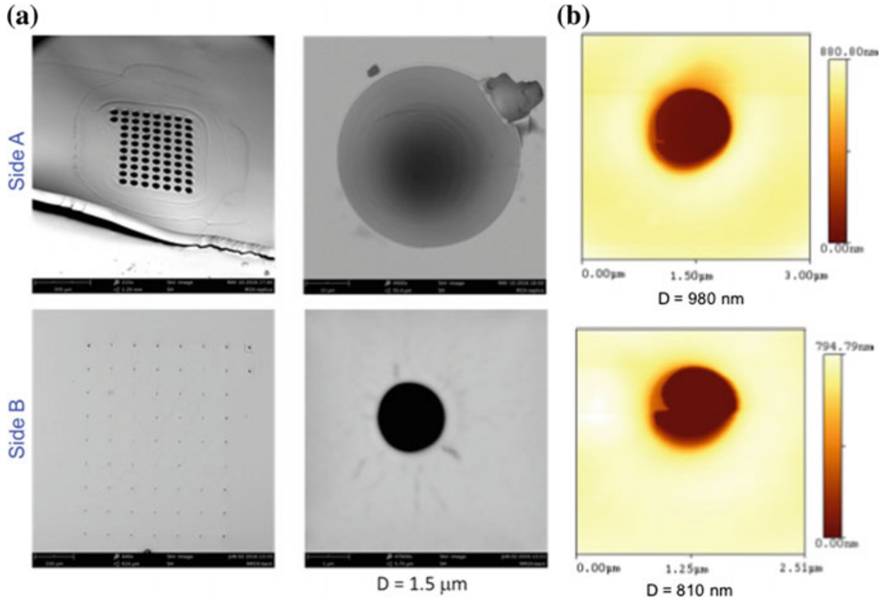

Fig. 12.4 Pores of PFPE membrane obtained by REM characterized by **a** SEM and by **b** AFM analysis

This process enabled to obtain membranes with a high fidelity to the master, due to a successful peeling of cured layers from the master, to the low surface tension and elastic modulus of PFPE-DMA. The step of spin-coating in the replica moulding process was needed to obtain thin layers of the viscous PFPE photoresist, but it also allowed controlling the thickness of the resist cured on the 2PP mould and to tailor the dimensions of the membrane pores. The membranes were finally characterized by SEM and AFM analysis. The results shown in Fig. 12.4 revealed the presence of pores with an average diameter of around 1 µm, thus demonstrating the successful exploitation of the proposed fabrication tool.

12.4.3 Developed Prototypes

The fabrication technology has been developed by optimizing the fabrication parameters of the tips on coverglass. The prototype has been later obtained by polymerizing a PFPE-PETA tip on top of a tipless commercial cantilever (AppNano HYDRA2R-200NTL). Usually, gold-coated cantilevers are employed for AFM measurements to increase laser intensity and thus enhance the detection of AFM probe movements. However, to avoid power absorption by the cantilever during laser irradiation, which would have led to material damage, an uncoated cantilever has been used. In order to

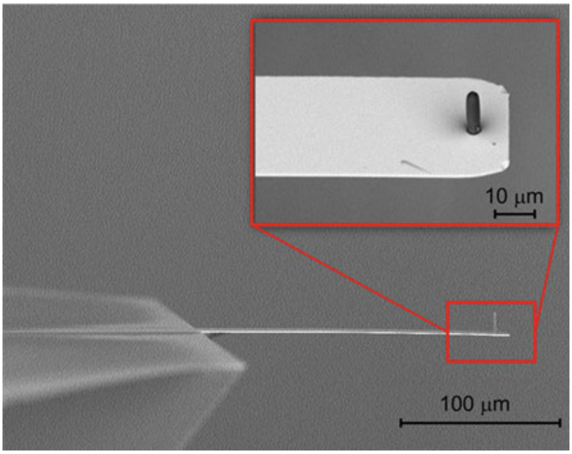

Fig. 12.5 SEM image of a PFPE-PETA AFM tip fabricated on top of a commercial cantilever [27]. The tip has a diameter of 3 μm and is 12.5 μm high

increase the adhesion between the resist and the Silicon Nitride cantilever, the latter has been silanized with 3-acryloxypropyl trimethoxysilane in vapour phase.

A sandwich support has been built to hold the cantilever and to allow the laser beam to reach the surface of the cantilever from above. A microscope slide has been covered with a layer of PDMS that could support the cantilever holder and two PDMS spacers. The spacers were holding a glass coverslip, so that the distance between the coverslip and the cantilever surface was more than the tip height, but less than the working distance of the objective used to focus the laser. The photoresist was inserted into the sandwich from the sides. The coverslip was necessary to ensure that the laser was always impinging on flat surfaces, and to avoid the transmission of the motion of the translation stages to the resist and to the cantilever.

The fabrication of the tip shown in Fig. 12.5 has been finally performed at 100 μm/s with a laser power of 15 mW. The distance between lines was set to 0.3 μm, and the spacing between planes to 0.1 μm. A one second stop between each plane has been introduced into the fabrication program to give the cantilever the time to stop vibrating after the vertical translation.

The cylindrical tip obtained after the fabrication on the cantilever is shown in Fig. 12.5, where it is possible to notice the hemispherical tip on top of it in the inset. The base diameter was measured to be 3 μm, and the tip was 12 μm high.

The uncoated cantilever caused the AFM feedback laser to give a very low signal during the prototype validation, hence a gold coating has been added by sputtering on the back of the cantilever after the tip fabrication. To prevent the gold from reaching the cantilever bottom surface and the tip, a mask has been realized via laser irradiation and chemical etching in fused silica. This enabled a precise insertion of the cantilever between two vertical walls, exposing only the desired surface to the gold target of the sputter coater.

12.5 Testing and Validation of Results

As mentioned in the previous section, new photoresists have been developed to realize hydrophobic stable AFM tips by 2PP. In particular, an optimal PFPE-based photoresist to be effectively structured by 2PP has been identified and examined in terms of chemical and physical properties. The exploitation of this photopolymer to successfully produce hydrophobic AFM tips by 2PP on commercial cantilevers has been demonstrated. In fact, AFM tips were validated by performing AFM indentations and by measuring the elastic modulus of medically relevant materials to be used as extracellular matrices in biological systems. Moreover, a clear understanding of tip-sample interactions exhibited by soft matter and hydrogels on the nanoscale were achieved, by comparing hydrophobic commercial probes and PFPE-based AFM tips.

12.5.1 Validation of Chosen Photoresist

A preliminary study was performed to investigate the chemical and physical properties of 6 PFPE photoresists, including commercially available grades. The PFPE-based resists used are different in terms of molecular weights and photo-reactive functionalities (Table 12.1). The exact chemical structure of PFPE photoresist is undisclosed apart from the PFPE-PETA resist, which was already synthesized and presented in a previous work by De Marco et al. [20]. All the PFPE-based resists showed a similar hydrophobicity, with a static contact angle versus water of 109–113° (Table 12.1) and a very low surface energy, suggesting the enrichment of a fluorine-based component on the resist surfaces.

In order to characterize the photo-reactivity of PFPE resists, the heats of crosslinking reaction were measured by a differential scanning calorimeter coupled with a UV source (UV-DSC), recording sample enthalpy changes when exposed to UV light. The results show that the heats of crosslinking for resists with methacrylate groups are lower than those with acrylates, probably due to the steric hindrance of methyl groups that decrease the reactivity of methacrylates during the polymerization [28, 29].

Among the photoresists with acrylate groups, a higher heat of crosslinking was obtained for PFPE-PETA with a value of 166 J/g (Table 12.1). This reveals that the resist with 6 acrylate groups is more reactive than others, suggesting why only PFPE-PETA provides reproducible and stable structures fabricated by 2PP, as shown in the previous paragraph. This finding also implies that it may be necessary to overcome a threshold value of heat of crosslinking to fabricate structures by 2PP, shedding light on mechanisms that makes photoresists suitable for 2PP structuring process. PFPE-PETA resist stands out as a suitable candidate for the microfabrication of hydrophobic and rigid AFM tips by considering also the relatively high shear modulus of 210 MPa measured for PFPE-PETA by dynamic mechanical analysis (DMA).

Table 12.1 PFPE -OH equivalent weights, total number and type of photo-reactive functionalities, enthalpies of crosslinking measured by differential scanning calorimetry coupled with a UV source (UV-DSC), water contact angles and surface energies for all the PFPE resists

Acronym	PFPE HO eq. weight	Photo-reactive groups per molecule	Enthalpy of crosslinking (J/g)	Water contact angle (°)	Surface energy (mN/m^2)
PFPE-DA	630	2 acrylates	111	n.a.[a]	n.a.[a]
PFPE-DUMA	630	2 methacrylates	55	110.0 ± 0.7	16.1 ± 0.5
PFPE-DUTA	1082	4 acrylates	116	112.9 ± 0.5	24.6 ± 3.9
PFPE-TUA	501	4 acrylates	111	112.6 ± 0.6	24.4 ± 4.0
PFPE-TUMA	636	4 methacrylates	69	112.8 ± 0.4	17.4 ± 1.4
PFPE-PETA	520	6 acrylates	166	108.9 ± 0.8	20.9 ± 0.5

[a] PFPE-DA resist solution was not homogeneous, providing a very rough surface where it was difficult to measure contact angles

12.5.2 Validation of Tailored Atomic Force Microscopy (AFM) Tips

The capability of 2PP fabricated tips to perform nanoindentation tests was validated by performing AFM measurements in air on two bio-medically relevant compliant materials, poly(dimethyl siloxane)-based elastomers and poly(ethylene glycol) hydrogels. The results obtained with 2PP fabricated tips were compared with those obtained using a commercially available probe with a spherical SiO_2 tip silanized with 1H, 1H, 2H, 2H perfluorooctyltriethoxysilane. As already shown in [30, 31], the functionalization of commercial probes with a fluorosilane is essential to decrease tip-sample interaction and perform AFM nanoindentations otherwise unfeasible on soft and hydrophilic materials in air.

Figure 12.6 shows force versus z-piezo displacement and force versus indentation curves obtained performing AFM indentation tests on PDMS 10:1 using a 2PP PFPE-PETA tip. A value of adhesion force of 6 nN was calculated as the difference between the minimum value of the force and the final value in the retraction curves measured with 2PP PFPE-PETA tips. This value of adhesion force is lower than the value obtained from curves measured using a fluorosilane-modified commercial tip (30 nN). This indicates that, using tips fabricated by 2PP structuring of PFPE-PETA photoresist, reduced tip-sample adhesive interactions occur in comparison with a fluorinated silicon tip, when samples like PDMS are tested. Moreover, an increase of maximum indentation ranges was achieved when using 2PP fabricated tips: from 35–45 nm obtained with a silanized commercial probe, to 60–75 nm using PEFE-PETA tips. This result demonstrates that PEFE-PETA photoresist enables the mea-

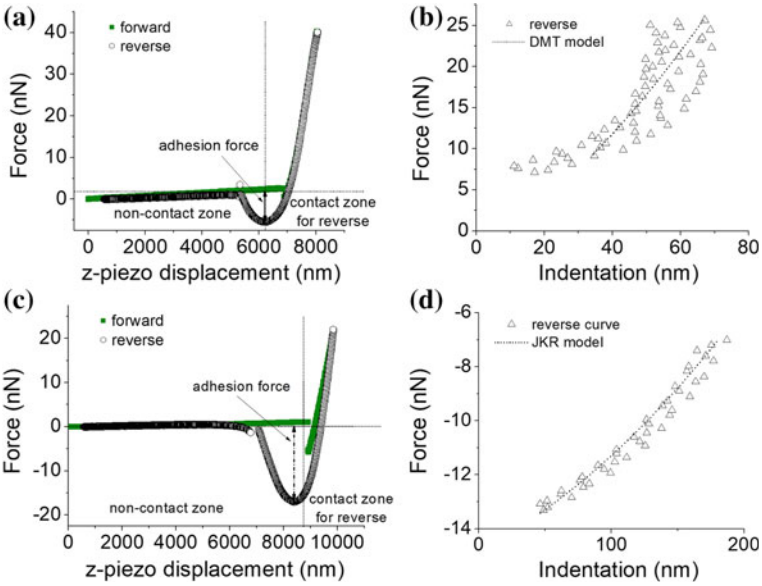

Fig. 12.6 Force versus displacement curves of **a** a PDMS 10:1 and **c** a PEGDA hydrogel samples measured with a AFM tip fabricated by 2PP with PFPE-PETA photoresist on a commercial cantilever. Force versus indentation curves obtained from reverse phase with superimposed **b** DMT and **d** JKR model curves in the dataset selected for the fitting for PDMS and for PEGDA, respectively [27]

Table 12.2 Maximum elastic indentation (δ), adhesion force (F_{ad}), Young's modulus (E_s) and reference Young's modulus values (E) for PDMS samples crosslinked with a prepolymer base/curing agent weight ratio of 10:1 (PDMS 10:1) and PEGDA hydrogels [27]

	Indentation δ (nm)	Adhesion force F_{ad} (nN)	Young's modulus E_s (kPa)	Reference value E (kPa)
PDMS 10:1	67 ± 6	5.9 ± 0.7	190 ± 191	1240 ± 50[a]
PEGDA hydrogels	195 ± 7	16.4 ± 0.5	31 ± 5	22 ± 2[b]

[a] Values measured by dynamic mechanical analysis

[b] Values derived from rheological measurements

surement of a large indentation range, leading to a better quality of elastic modulus prediction from force-distance curves.

Young's modulus of PDMS was determined by employing Derjaguin-Müller-Toporov (DMT) model, suitable for soft samples with a low surface energy [32]. A value of elastic modulus of 1.2 ± 0.2 MPa was obtained and found to be in good agreement with reference values measured by conventional techniques such as DMA (Table 12.2) [27].

Nanoindentation tests using 2PP fabricated tips were also performed on crosslinked poly(ethylene glycol) hydrogels prepared by UV polymerization of a poly(ethylene glycol) diacrylate (PEGDA, 10% wt.) and water (90% wt.), because these materials are increasingly studied and selected to act as artificial extracellular matrices. Figure 12.6c, d show force versus displacement and force versus indentation curves measured on PEGDA hydrogels. PEGDA hydrogels exhibit an adhesion force higher than PDMS samples 10:1 in the unloading curves (see Table 12.2). By using a commercial tip with a fluorosilane coating, an adhesion force of 36.7 ± 0.1 nN was obtained on PEGDA hydrogels. The higher hydrophobicity of 2PP fabricated tips led to a higher value of adhesion force with respect to the 2PP PFPE-PETA tips (16.4 ± 0.5 nN). Therefore the 2PP PFPE-PETA tips are more effective than fluoro-silanized tips at reducing adhesive interaction. Using the 2PP fabricated tips, a larger indentation range of 195 ± 5 nm was measured when compared to fluoro-silanized SiO_2 probes (120 ± 18 nm), thus resulting in a more accurate calculation of the elastic modulus also for PEGDA hydrogels. The JKR model was selected to calculate Young's modulus of hydrogels, because this model is more appropriate than DMT model for soft samples with a high surface energy [33]. Only unloading curves were considered for the evaluation of elastic moduli to neglect any dissipative phenomena, thus leading to an average value of 31 ± 5 kPa. The obtained value of modulus matches the average value measured using standard tips (32 ± 6 kPa) and it is also very similar to the average value of modulus estimated from rheological measurements (22 kPa). These findings confirm the ability of 2PP tips to perform nanoindentation tests in air on soft samples with very high water content and to measure their Young's modulus [27].

12.6 Conclusions and Future Research

The results of this work demonstrated that the 3D prototyping capability of 2PP technology is suitable for the realization of specific products following the customer needs. In fact, the results showed that this technology is suitable to fabricate AFM tips with customized geometries, mechanical and chemical properties. This can be easily achieved thanks to the 2PP capability of efficiently nanostructuring different photopolymers. Preliminary results on the realization of porous membrane by replica moulding of a 2PP fabricated master showed the possibility to scale up the fabrication process for an example customizable product.

In particular, the obtained results cover different aspects ranging from the development of a new photoresist [33] to the optimization of the laser fabrication protocols for an efficient structuring of the developed photoresist, to the fabrication of AFM tips on commercial cantilevers and to their experimental validation [27]. A PFPE-based photoresist has been indeed developed to fabricate hydrophobic and rigid AFM tips on commercial cantilever. The chosen tip geometry is suitable for nanoindentation measurements on soft materials and the realized prototypes have been used to characterize the elastic modulus of biologically relevant materials as PDMS and PEGDA hydrogels. The tips demonstrated to have low adhesion interactions with the sample surface thanks to the high hydrophobicity of the chosen polymer. This feature permitted to characterize the sample by measuring large indentation ranges, thus enabling one to retrieve the elastic modulus from the force-distance curves with higher precision. The obtained results, in comparison with fluorosilane-functionalized commercial tips, demonstrated that the hydrophobic PFPE tips fabricated by 2PP can be used for nanoindentation test. Indeed, these showed a better performance in combination with the advantage of being tailor-made for samples under investigation in terms of tips shape and dimension.

The positive results give birth to new opportunities to further explore the customization offered by two-photon polymerization and replica moulding. The functionalization of the tip allows testing biological and functional surfaces and the development of AFM micromechanical testing models and protocols for soft and biological surfaces. Moreover the development of specialty photoresists with tailored surface properties would open the possibility to use 2PP fabricated tips for chemical sensing. Further work can be also focused on the membranes in order to achieve smaller pore and validate them for some applications, e.g. integrating them in microfluidic chips for water filtering analysis.

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