Construction of three-dimensional biomolecule structures employing femtosecond lasers

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The authors demonstrate here a method for three-dimensional patterning of proteins and other biological molecules. The method employs femtosecond-laser-induced three-photon polymerization, a technique which enables the construction of arbitrary two- and three-dimensional structures of submicron resolution. Biotin is subsequently attached to the three-dimensional (3D) structures via UV-activated cross-linking. The integrity of the photolytically immobilized biotin is confirmed by detecting the binding of fluorescently labeled avidin via fluorescence microscopy and via a surface acoustic sensor technique. In all, the technique opens the way for the fabrication of structures with a wide range of biomaterials as well as studying their dynamics within complex 3D structures.


The analysis of biological samples with biosensors has been the focus of much analytical research in recent years. Micropatterned surfaces have become increasingly important to biosensor systems, with patterned arrays of antibodies, DNA, enzymes, and supported lipid membranes proposed to monitor the functions of biomolecules and cells in situ.1–4 The construction of arrays of micrometer dimension allows the measurement of many different biological analytes using samples of very small volume. Furthermore, such structures enable the investigation of the dynamics of biomaterials in scales typical of the confined cytoplasm and nucleus.5

A number of different approaches have been examined for fabricating patterned biological polymer surfaces,6–9 photoactivation of light sensitive molecules being one of them.9,10 The use of photoactive biotin derivatives is an attractive technique, as it not only offers the exploitation of a strong noncovalent biological interaction between biotin and the glycoprotein avidin but also it allows binding of biotinylated ligands, enabling the fabrication of multifunctional assemblies of molecules upon the sample surface.3,11

Almost in all cases, biomolecule patterning has been two dimensional (2D). Three-dimensional (3D) patterning on the basis of DNA complex structures has been demonstrated,12 but requires extensive biochemical facilities that are not available except to specialized laboratories. We demonstrate a method of 3D biotin-avidin patterning which enables the construction of arbitrary two- and three-dimensional shapes, not restricted to array-based shapes. By going from 2D to 3D one can increase greatly the active surface area and thus the sensitivity of the system without sacrifices to the size of the sensor. The method allows not only prototyping but direct sensor construction.

We construct 3D structures via three-photon polymerization of the biocompatible hybrid glass ORMOCER13 by a 200 fs duration, 1.028 μm wavelength Yb laser. Biotin is subsequently immobilized on the surface of the structures by excimer laser photoactivation of photobiotin and further exposed to fluorescently labeled avidin. Fluorescence micros-
copy is used to visualize the distribution of fluorescent avidin. In order to examine the binding of streptavidin to biotin, a surface acoustic wave (SAW) device is used. The specificity of the binding is demonstrated by confirming the non-binding of streptavidin when exposed to non-biotin-treated ORMOCER surfaces.

For the fabrication of the 3D nanostructures, we use the biocompatible organic-inorganic hybrid ORMOCER (Micro Resist Technology), which is characterized by high transparency in the visible and near infrared ranges above 400 nm (Refs. 13 and 14) and therefore allows three-photon polymerization using a 1028 nm laser. Polymerization is initiated by the reaction of the radical photoinitiator Irgacure™ 369. Photobiotin, Atto 565-streptavidin, and poly(methylmethacrylate) (PMMA) are obtained from Sigma Aldrich. Biotinylated antihuman IgG(H+L) antibodies are obtained from Vector Laboratories.

The construction of 3D structures has been carried out using nonlinear optical stereolithography, a technique that allows the fabrication of three-dimensional structures with submicron resolution. The technique has been used with a variety of acrylate and epoxy materials15,16 for the fabrication of and several components and devices such as photonic crystal templates14,17 and micromechanical devices.18–20 The employed experimental setup and procedure has been described in detail elsewhere.21

Figure 1 shows the scanning electron microscopy image of a structure built using this technique. It consists of five step-in-squares, which serve the purpose of building a robust support structure and four vertical and horizontal lines, which serve as dividers. After building, the samples were inspected in a fluorescence microscope, and some of them were found to fluoresce in the green. Further investigations showed that this fluorescence is only observed from the samples made with ORMOCER that is more than six month old. This fluorescence is most likely due to degradation products of the photoinitiator. For all the structures fabricated in study, only fresh ORMOCER has been used and control samples made to confirm that there is no fluorescence from the base material.

For biomolecule patterning, the 3D components are covered with a photobiotin solution consisting of 1 mg of photobiotin in 1 ml distilled water and 1 ml ethanol. The solvent is removed by drying the samples under vacuum at room temperature in the dark for 1 h. Subsequently, the samples are exposed to 20 pulses, 1 Hz repetition rate UV radiation from an excimer KrF laser (TUI Braggstar, 248 nm wave-
pulses of UV radiation of a 248 nm KrF excimer laser biotinylated surface of the acoustic sensor is exposed to 20 

tering experiments, mass adsorbed to the polymer surface can be monitored in real time by following the phase change of the wave during the adsorption process.

The SH-SAW device is fabricated on a 0.5 mm thick piezoelectric quartz crystal. The interdigital transducers (IDTs) comprise of 10/200 nm thick chromium/gold electrode and consist of 80 pairs of split fingers with a periodicity of 45 μm. A 1.2 μm thick waveguide layer of PMMA is deposited on the surface of the acoustic device by spin coating and baked subsequently at 195 °C for 2 h to facilitate solvent evaporation. The operating frequency of the acoustic waveguide device is 105 MHz.

In order to construct a thin film of ORMOCER on the device surface, 25 μl of a 1 μg/ml ORMOCER/4-methyl-2-pentanone solution is spin coated on the PMMA surface and subsequently dried under an UV lamp for 30 min. 130 μl of photobiotin solution in different concentrations was applied to the ORMOCER-coated surface of the acoustic sensor. After drying in a vacuum oven for 1 h at room temperature, the biotinylated surface of the acoustic sensor is exposed to 20 pulses of UV radiation of a 248 nm KrF excimer laser (TUI Braggstar).

The phase of the output electrical signal with respect to reference signal data is measured by a Hewlett Packard 4195A network analyzer interfaced to a personal computer using LABVIEW software. A Perspex flow cell with a silicone rubber gasket is used to hold the solution in place over the device surface, 25 

 binding occurs when the ORMOCER is treated with a photobiotin concentration larger than 100 μg/ml. This is related mainly to the packing ability of avidin which, in turn, is affected by the density of the bound photobiotin.

Finally, the SAW sensor technique has been used to examine the attachment of the biotinylated antihuman IgG(H+L) antibody. A permanent phase change is again measured, indicating the functional operation of the photobiotin-avidin complex.

To conclude, we have demonstrated a fabrication method of 3D biopolymer structures by exploiting recent advances in materials processing by femtosecond-laser technology. The method is straightforward and can provide the basis for developing a wide variety of biopolymer structures by exploiting the binding capabilities of the biotin-streptavidin system. As compared with 2D lithographic approaches, it offers the advantage of producing a high active area in a small size, thereby enabling much higher detection sensitivity.

Another interest for developing these structures is that they constitute ideal models where processes such as ion and biopolymer transport through confined spaces can be studied.5

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