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A Photoresist with Low Fluorescence for Bioanalytical Applications

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Abstract

The negative photoresist SU-8 has found widespread use as a material in the fabrication of microelectrical-mechanical systems (MEMS). While SU-8 has been utilized as a structural material for biological MEMS, a number of SU-8 properties limit its application in these bioanalytical devices. These attributes include its brittleness, nonspecific adsorption of biomolecules, and high fluorescence in the visible wavelengths. In addition, native SU-8 is a poor substrate for cellular adhesion. Photoresists composed of resins with epoxide side groups and photoacids were screened for their ability to serve as a low fluorescence photoresist with sufficient resolution to generate microstructures with dimensions of 5-10 μm. The fluorescence of structures formed from 1002F photoresist (1002F resin combined with triarylsulfonium hexafluoroantimonate salts) was as much as 10 times less fluorescent than similar SU-8 microstructures. The absorbance of 1002F in the visible wavelengths was also substantially lower than that of SU-8. Microstructures or pallets with an aspect ratio as high as 4:1 could be formed permitting 1002F to be used as a structural material in the fabrication of arrays of pallets for sorting adherent cells. Several different cell types were able to adhere to native 1002F surfaces and the viability of these cells was excellent. As with SU-8, 1002F has a weak adhesion to glass, a favorable attribute when the pallet arrays are used to sort adherent cells. A threshold, laser-pulse energy of 3.5 μJ was required to release individual 50-μm, 1002F pallets from an array. Relative to SU-8, 1002F photoresist offers substantial improvements as a substrate in bioanalytical devices and is likely to find widespread use in BioMEMS.

Introduction

Negative photoresists are composed of a resin, a photoactive compound, and a solvent.[1,2] When illuminated with light, the resin is converted to an insoluble form typically by undergoing an increase in molecular weight or by formation of a new insoluble molecule. The increase in molecular weight is generally achieved by polymerization or cross-linking of the resin monomers. Insolubility can also be attained by initiation of a photochemical reaction to alter polarity or hydrophobicity. For polymerization and cross-linking reactions, the photoactive compound is generally a photoinitiator which forms free radicals or strong acids upon exposure

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to light. Chemically amplified negative photoresists take this process one step further. Light is used to generate a chemical catalyst initiating a cascade of events, thus amplifying the effects of the initial photon absorption.[3,4] Chemically amplified resists possess high sensitivity to UV light and provide high resolution structures. These resists have found a multitude of uses, for example in image reversal, as a protective layer during etching, and in formation of high aspect ratio microstructures.[5]

SU-8 photoresist was introduced in 1989 by IBM as an chemically amplified, negative photoresist.[1,6] SU-8 photoresist is comprised of the SU-8 resin (bisphenol-A novolac epoxy resin, CAS 28906-96-9), the photoinitiator triarylsulfonium hexafluoroantimonate salts, and a solvent, typically γ-butyrolactone (GBL) or cyclopentanone. In addition to its use as a high-contrast negative photoresist, SU-8 also possesses a number of properties that make it attractive as a structural material in microfabricated devices. SU-8 is biocompatible, rigid, thermally and chemically stable, and transparent to light above 360 nm.[1,7-11] SU-8 has been employed as a structural component in microelectrodes, atomic force microscopy tips, electrophoresis microchips, and other applications.[12-22] While SU-8 has many assets, it has significant weaknesses when used as a structural material for bioanalytical applications. SU-8 is brittle and fractures easily.[23,24] Native SU-8 is hydrophobic and prone to nonspecific adsorption of bioanalytes.[25-28] In addition, most biological cells will not attach to native SU-8. Potentially the greatest weakness of SU-8 though is its high fluorescence in the visible wavelengths.[17] Since fluorescence is the preferred detection method for many bioassays, the very high background fluorescence generated by SU-8 has limited its utility as a substrate in devices for biological studies.

A successful application of SU-8 as a structural component in a bioanalytical device is its use to form arrays of pallets to separate adherent cells.[29] The pallet-array system permits cells or colonies of cells to be sorted while they remain on their growth surface broadening available selection criteria for the cells/colonies. The high aspect ratio of SU-8 allows a wide range of pallet heights (25-100 μm) and sizes (20-500 μm) to be constructed. Due to the good mechanical strength of SU-8, pallets dislodged from a glass surface using the focused beam of a pulsed laser remain intact.[30] The optical transparency of SU-8 has permitted a number of cell selection strategies to be applied to cells cultured on the SU-8 pallet arrays. These assays include selections utilizing transmitted light microscopy and fluorescence microscopy, particularly in the red wavelengths.[31,32] Fluorescence detection in the green is also possible, but at greatly reduced sensitivity due to SU-8's high background fluorescence at these wavelengths. A second weakness of the SU-8 pallets is that they must be coated with adsorbed layers or covalently attached molecules before most cells will attach and grow on the pallet surface.[31] A negative photoresist with decreased fluorescence and enhanced cell adhesive properties would broaden the use of the pallet arrays to applications requiring highly sensitive fluorescence detection and to separation of a wider range of cell types.

In the current work, we surveyed a variety of resins and photoinitiators to develop a low fluorescence alternative to SU-8 photoresist. The fabrication process for the new 1002F photoresist was optimized including the spin-coating parameters, soft-bake times and temperatures, UV illumination time, and post-exposure baking procedure. In addition, the emission spectra as well as the absorbance at varying wavelengths was measured and compared to that of SU-8 and poly(dimethylsiloxane) (PDMS). Furthermore, the fluorescence of the 1002F photoresist was compared to that of SU-8 using fluorescence microscopy filters typically used to view cellular fluorescence. The aspect ratio for the formation of arrays of pallets was defined as well as the adhesion and viability of cells on native 1002F photoresist. The ability of the 1002F pallet arrays to support virtual walls which are required to localize cells to the pallet tops was characterized. Since the ultimate goal for the new photoresist was as a structural material for pallet arrays for cell sorting, the energy required to release 1002F pallets from an

array using a focused, pulsed laser was measured. These studies demonstrate the utility of the new 1002F photoresist for the fabrication of arrays of pallets for use in the pallet array system.

Experimental Section

Materials

UVI-6976 photoinitiator (triarylsulfonium hexafluoroantimonate salts in propylene carbonate) was purchased from Dow Chemical (Torrance, CA) and poly(dimethylsiloxane) (PDMS) (Sylgard 184 silicone elastomer kit) was purchased from Dow Corning (Midland, MI). SU-8 photoresist and SU-8 developer (1-methoxy-2-propyl acetate) were obtained from MicroChem Corp. (Newton, MA). EPON resin 1002F (phenol, 4,4′-(1-methylethylidene)bis-, polymer with 2,2′-[(1-methylethylidene) bis(4,1-phenyleneoxymethylene]bis[oxirane]) was obtained from Miller-Stephenson (Sylmar, CA). All other photoinitiators and resins were from Sigma-Aldrich (St. Louis, MO) as were γ-butyrolactone (GBL), L-glutamine and poly(D-lysine)hydrobromide (MW 70,000-150,000). (Heptadecafluoro-1,1,2,2-tetrahydrodecyl)trichlorosilane was from Gelest Inc. (Morrisville, PA). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin were obtained from Invitrogen (Carlsbad, CA). Cell proliferation kit II (XTT) was obtained from MD Biosciences Inc. (St. Paul, MN). Silicone O-rings (24 mm outer diameter) were purchased from McMaster-Carr (Los Angeles, CA). All other reagents were obtained from Fisher Scientific (Pittsburgh, PA).

Fabrication of Films and Pallets from SU-8 or 1002F Photoresist

1002F-50 photoresist was made by dissolving EPON resin 1002F and triarylsulfonium hexafluoroantimonate salts in GBL at a ratio of 61% 1002F resin: 6.1% photoinitiator: 32.9% solvent (weight percentage). 1002F-10 photoresist was made of the same components but at a ratio of 49% 1002F resin: 4.9% photoinitiator: 46.1% solvent. SU-8 and 1002F films and pallets were fabricated on pre-cleaned glass slides ($75 \times 25 \times 1$ mm^3). Films and pallets were fabricated in a manner similar to that previously described.[29-31] SU-8 films with different thickness (10, 25, 50, 75, and 100 μm) were obtained by spin-coating SU-8 photoresist on glass slides following the protocol provided by MicroChem Corp.[1,33,34]

To fabricate films and pallets composed of 1002F, 1002F films of different thickness (10-100 μm) were obtained by spin coating 1002F-50 or 1002F-10 resist on a glass slide in a two step process as shown in Table 1. The initial spin was for 10 s followed by a second spin for 30 s. The coated slides were then soft baked on a hotplate at 65°C as shown in Table 1, followed by a second bake at 95 °C for the time indicated in Table 1 to remove organic solvent. After baking, the slides were allowed to slowly cool to room temperature on the hotplate (~30 min). To prepare an array of pallets, the 1002F film was exposed to UV light through a photomask with the appropriate design features for the times indicated in Table 1 using a collimated UV source (6 mW/cm^2, Oriel, Newport Stratford, Inc., Stratford, CT). The post-exposure bake was performed on a hotplate at 65°C followed by 95°C with the time at each temperature optimized for the thickness (Table 1). After cooling to room temperature, the 1002F samples were developed in SU-8 developer, rinsed with 2-propanol, and dried in a stream of nitrogen.

Collection of Fluorescence Emission Spectra

SU-8 or 1002F photoresist or resin was poured into a mold (3.5 mL, $1 \times 1 \times 3.5$ cm) made out of PDMS. The mold with photoresist or resin was placed in a vacuum oven at 100°C for 5 days to completely remove organic solvent. The mold and contents were then slowly cooled to room temperature. Spectra were then recorded from the sample blocks. The blocks were excited at 485 nm with a ±2 nm band pass and the fluorescence measured from 490 to 650 nm with a ±2 nm band pass using a SpectraMax M5 microplate reader (Molecular Devices Corporation,

Sunnyvale, CA). Data points were taken at 10 nm intervals. Fluorescence emission from cured blocks of PDMS was measured in a similar manner.

Qualitative Characterization of SU-8 Fluorescence with Standard Microscopy Filter Sets

Patterned SU-8 lines (100-μm width, 500-μm inter-line distance) of different heights (10, 25, 50, 75, 100 μm) were fabricated on glass slides. SU-8 lines were then examined using a Nikon Eclipse TE300 inverted fluorescent microscope equipped with three fluorescent filter sets: a fluorescein filter set (B-2A, Nikon Instruments, excitation filter 450-490 nm, dichroic 500 nm long pass, emission 520 nm long pass), a tetramethyl rhodamine filter set (G-2E, Nikon Instruments, excitation filter 528-553 nm dichroic 565 nm long pass, emission 590-650 nm), and a Cy5 filter set (41008, Chroma Technology, excitation filter 590-650 nm, dichroic 660 nm long pass, emission 665-740 nm). A microscope objective (Nikon Plan Fluor, 20X, N.A. 0.50) was used to collect the fluorescent light which was detected using a cooled CCD camera (Photometrix Cool Snap *fx*, Roper Scientific, Tucson, AZ) controlled by Metafluor software (Molecular Devices, Sunnyvale, CA). For fluorescence imaging of cells, the signal-to-noise ratios of cells cultured on pallet arrays were determined using standard image analysis software (ImageJ, NIH).

Measurement of SU-8 and 1002F Absorption

To measure the absorbance of SU-8 and 1002F photoresists, films (250 μm) were fabricated on a glass slide. The light absorption of the samples was measured with a JASCO V-530 UV-Vis spectrophotometer (Jasco Inc, Easton, MD) using air as a blank.

Measurement of Cell Metabolism

Rat basophilic leukemic (RBL) or human glioblastoma (A172) cells were cultured in a 37°C, 5% CO_2 incubator at a density of 50,000 cells/mL (100 μL) in chambers with bases constructed of glass, SU-8, or 1002F. To measure cell metabolism, XTT assays were performed on the cells at varying time points as per the manufacturer's instructions (Roche Applied Science, Indianapolis, IN). Cells from four separate chambers were assayed at each time point. The XTT assay measures the ability of cells to metabolize XTT (sodium 3′-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate) to a colored product. The presence of the formazon product was determined by measuring the absorbance at 480 nm (SpectraMax M5, Molecular Devices Corporation, Sunnyvale, CA).

Laser-Based Pallet Release

Release of pallets by a single pulse (5 ns, 532 nm) from a focused Nd:YAG laser (ACL-1, New Wave Research, Fremont, CA) was performed as described previously.[30] Curves of the probability of pallet release with respect to the energy of the laser pulse were also constructed as described previously.[30] The threshold energy for pallet release (energy at which 50% of the pallets released) was determined by fitting these curves to a Gaussian error function.

Results and Discussion

Identification of Candidate Photoresists

SU-8 photoresist exhibits substantial fluorescence in the visible wavelengths when excited with blue light (Fig. 1). Both SU-8 resin and the photoinitiator, triarylsulfonium hexafluoroantimonate salts possess complex ring structures that may act a source of this fluorescence (See supplemental data). To determine if either the resin or photoinitiator was the dominant source of the fluorescence, the fluorescence of the resin alone was measured (Fig. 1). When excited at 480 nm, SU-8 resin was still fluorescent although 3 times less so than the

photoresist at 540 nm. For comparison SU-8 photoresist is 800 times more fluorescent than PDMS at the same wavelengths. SU-8 resin was also less fluorescent than SU-8 photoresist at other wavelengths. However both the SU-8 resin and photoinitiator (or its decomposition products) acted as sources of fluorescence in the photoresist.

To determine whether a photoresist with similar properties to SU-8 but with lower fluorescence could be developed, a panel of photoinitiators and resins were screened for their ability to form microstructures of low fluorescence (Table 2 in the Supplementary Information Section). The photoinitiators were selected to be representative of both ionic photoacid generators (diaryliodonium and triarylsulfonium salts) and nonionic photoacid generators (N-hydroxyimide sulfonates and others). The resins selected were solids at room temperature and possessed cross-linkable epoxy groups. The tested resins were poly(ethylene-co-glycidyl methacrylate), poly(ethylene-co-methyl acrylate-co-glycidyl methacrylate), tetraphenylolethane glycidyl ether, poly[(phenyl glycidyl ether)-co-dicyclopentadiene], and EPON 1002F resin (bisphenol A/epichlorohydrin-based epoxy resin) in addition to the SU-8 resin. Mixtures of the different combinations of photoinitiators and resins were spin-coated onto a glass or quartz surface. After an initial soft bake to remove the solvent, the mixtures were illuminated with UV light through a test mask. After a post-exposure bake, the sample was developed. The samples were assessed for their ability to act as a photoresist in the fabrication of microstructures as well as for the intensity of their fluorescence in the visible wavelengths. Of the tested mixtures, only the 1002F resin with triarylsulfonium hexafluoroantimonate salts yielded high quality microstructures with a lower fluorescence than that of the SU-8 resin (Fig. 1,2).

Characterization of 1002F Resin with Triarylsulfonium Hexafluoroantimonate Salts (1002F Photoresist) for the Fabrication of Pallets

To further characterize the ability of 1002F photoresist to act as a substrate for microstructures, a variety of different sized square and circular pallets with heights and diameters of 5-300 μm and ≥5 μm, respectively were fabricated. For each height, the ratio of the resin to photoinitiator in the resin was optimized as well as each of the fabrication steps (Table 1). When fabricated using these conditions, the pallets possessed the appropriate shapes with crisp edges by light microscopy (Fig. 2A). Pallets as small as 5 μm could be fabricated (Fig. 2D). To view the side walls of the pallets, the structures were imaged by scanning electron microscopy. The side walls appeared smooth by SEM (Fig. 2B). For these pallets, the aspect ratio (height:width) was determined by fabricating structures with theoretical aspect ratios from 1:1 to 5:1. Ratios of 4:1 (Fig. 2C) and lower were successfully fabricated, but structures of 5:1 were not successfully formed. For comparison the maximal aspect ratio for SU-8 pallets was 10:1 using similar fabrication conditions. The aspect ratio of the 1002F photoresist, however, was sufficient for the fabrication of pallets of a wide range of sizes for cell sorting applications.

Fluorescence of 1002F Photoresist and Resin

Since 1002F photoresist did possess fluorescence, it was important to identify the source of the fluorescence. A block of 1002F resin was illuminated at 485 nm and the fluorescence at longer wavelengths measured and compared to that of an identical sized block of 1002F photoresist. In the visible wavelengths, the 1002F resin by itself was significantly less fluorescent than the 1002F photoresist (Fig. 1). At an emission wavelength of 540 nm the photoresist was over 5 times more fluorescent than the resin. These results suggested that the photoinitiator, triarylsulfonium hexafluoroantimonate salts, or its breakdown products were responsible for the majority of the 1002F photoresist fluorescence. To further decrease the fluorescence of structures composed of 1002F, new low fluorescence photoinitiators will be required.

Sorting biological cells from a mixture of cells is frequently performed by labeling the cells with a fluorescent reagent and then selecting those cells with the appropriate level of fluorescence. Therefore, it is important to understand the fluorescence properties of the 1002F pallets using excitation and emission wavelengths commonly employed in fluorescence microscopy. Pallets of varying thickness were fabricated from SU-8 or 1002F photoresist and their fluorescence measured using filter sets designed for fluorescein, tetramethyl rhodamine, and Cy5 (Fig. 3A-C). As expected thicker pallets of SU-8 or 1002F exhibited greater fluorescence than thinner pallets. For all pallet thicknesses and excitation/emission wavelengths, the SU-8 pallets were substantially more fluorescent than the 1002F pallets. When excited at blue wavelengths, SU-8 pallets 50 μm or greater in thickness possessed over an order of magnitude more fluorescence than the 1002F pallets (Fig. 3A). As the excitation/emission wavelengths increased, the absolute fluorescence of both SU-8 and 1002F decreased. For green excitation wavelengths, the 1002F pallets were as much as 10 times less fluorescent than SU-8 (Fig. 3B). When illuminated with red light, the 1002F pallets were nearly nonfluorescent while the SU-8 pallets continued to display significant fluorescence (Fig. 3C). These data demonstrate that 1002F is a superior substrate for pallet fabrication when fluorescence is used for detection.

Absorbance of 1002F Photoresist

Many bioapplications using photoresists as a structural material employ absorbance measurements for analyte detection. For this reason, films of SU-8 or 1002F photoresist (250 μm thick) were coated on a slide. The films were exposed to UV light (without a photomask) and baked as described in the methods section. The absorbance of the films was then measured. SU-8 and 1002F possessed high transmittance at long wavelengths (500-600 nm) (Fig. 3D). Between 400 and 500 nm, the absorbance of SU-8 increased as the wavelength decreased whereas 1002F remained nearly transparent. By 400 nm, the absorbance of SU-8 was greater than twice that of 1002F. The absorbance of both photoresists increased rapidly as the wavelength decreased below 400 nm; however, at 370 nm the absorbance of 1002F remained half that of SU-8. By 360 nm, both photoresists possessed an absorbance greater than 1. Neither SU-8 nor 1002F are good candidates as structural elements for measurements of absorbance in the ultraviolet wavelengths. However for wavelengths greater than 400 nm, 1002F has excellent potential and is superior to SU-8 as a structural material for applications utilizing absorbance as a detection method. Other potential applications suitable for 1002F include the fabrication of miniaturized, transparent, optical elements such as lenses.

Cell Growth on 1002F Films

Since cells have difficulty attaching to bare SU-8, coatings must be covalently attached or adsorbed onto the SU-8 surface in order for cells to attach. To assess the ability 1002F photoresist to support cell adhesion, RBL and A172 cells were cultured on 1002F films placed on glass. Within 2-3 hours, cells were observed to attach and begin spreading on the 1002F surface (data not shown). Other tested cells (rat fibroblast (208F) and human cervical carcinoma (HeLa)) behaved similarly.

To assess their longer term health, RBL and A172 cells were cultured on glass, SU-8, or 1002F surfaces for varying times and then assayed for their ability to metabolize XTT. In this assay, metabolically active cells convert XTT, a yellow tetrazolium salt, to an orange formazon product. Similar results were obtained for both cell types on the three different surfaces suggesting that none of the materials were toxic to these cells (Fig. 4A,B). The cells cultured on SU-8 possessed slightly slower XTT metabolism than those on glass or 1002F. Most likely this was due to the poor adhesion of cells to the SU-8 surfaces until the SU-8 became fully coated with adsorbed proteins from the tissue culture medium. These results suggest that many cell types will be able to adhere to and grow on surfaces composed of native 1002F.

Cell Growth on Arrays of 1002F Pallets

For arrays of 1002F pallets to serve as a suitable format for cell selection and sorting, cells must attach to and grow on the top surfaces of the pallets and not on the glass surface between the pallets. A successful strategy to direct cells to the top surface of SU-8 pallets is to coat the array with a hydrophobic silane.[29,30] Upon immersion of the SU-8 pallet array in an aqueous medium, air is stably trapped between the pallets forming a virtual wall between the pallets. These virtual walls exclude cells from the region between the pallets forcing the cells to attach to the top surfaces of the pallets. To determine whether 1002F pallets could support virtual walls, an array of 1002F pallets was coated with a perfluoroalkylsilane layer ((heptadecafluoro-1,1,2,2-tetrahydrodecyl)trichlorosilane).[29] The array was then coated with collagen and immersed in an aqueous solution. Air trapped between the pallets was readily visualized as was the air:water interface at the edge of the pallet array (data not shown). To insure that these air walls could be used to localize cells to the pallet top surfaces, HeLa cells were cultured on the 1002F pallet array with virtual walls. Of 109 HeLa cells visualized, 100% were located on the pallet top surface with 0% on the intervening glass surfaces (Fig. 5A). Similar results were obtained for a collagen-coated, SU-8 array with virtual walls and cultured HeLa cells (Fig. 5B).

To assess the viability of the cells cultured on the 1002F array, the cells were loaded with viability indicator, Oregon Green diacetate. Living cells metabolize the dye to its counterpart, Oregon Green, which exhibits a green fluorescence. All cells on the 1002F array metabolized and retained the dye demonstrating that they were viable (Fig. 5C). For comparison, RBL cells loaded with Oregon Green on an SU-8 array are also shown (Fig. 5D). The most notable difference between the images of the SU-8 and 1002F arrays is the high background fluorescence of the SU-8 pallets compared to the nonfluorescent, 1002F pallets. The signal-to-noise ratio was 2.7 for cells on SU-8 pallets, and 8.2 for cells on 1002F pallets. These results demonstrate that the 1002F photoresist can be used to form arrays of cells at known locations and with low background fluorescence.

Laser-based release of 1002F pallets from arrays

A key aspect of the SU-8 pallet arrays is that individual pallets can be released by a single focused pulse from a Nd:YAG laser (5 ns, 532 nm, 2 μJ). On-demand, single-pallet release permits the collection of pallets possessing a desired cell and, consequently, the separation of that cell from the mixture of cells on the array. Thus, for 1002F to be successful as a substrate for a low-fluorescence pallet array, the 1002F pallets must be readily releasable from the array using low pulse energies. To ascertain whether 1002F pallets were releasable, single pallets (50 μm side and height) in an array were targeted for release. When the laser pulse energy was greater than 4 μJ, 100% (n=50) of the 1002F pallets were released by a single pulse focused at the interface of the glass:1002F interface. None of the adjacent, untargeted pallets were released. To determine the threshold energy for pallet release, the probability of releasing a pallet was measured at varying pulse energies. The threshold energy for 1002F pallet release was 3.5 μJ. For comparison the threshold energy for identical-sized SU-8 pallets was 2.0 μJ. While the energy for 1002F pallet release was greater than that for SU-8 pallets, successful release and collection of living cells on SU-8 pallets has been performed with laser release energies up to 10 μJ.[32] Thus, the 1002F pallets should be suitable for sorting many different types of cells.

There are several possible reasons for the higher energies required to detach the 1002F pallets from the underlying glass surface relative to that for the SU-8 pallets. 1002F has only two epoxide groups per monomer unlike SU-8 which possesses 8 epoxides per monomer. Thus, while the SU-8 polymer is extensively crossed linked, 1002F is a linear, uncrossed-linked polymer. As a result 1002F is a softer, more flexible material than SU-8. This difference is also

observed when films of 1002F and SU-8 are fabricated (data not shown). The softer, more flexible 1002F may absorb more mechanical energy before the contact with the glass is disrupted. In addition, these features may also afford 1002F with a greater number of molecular interactions with the glass surface permitting a tighter bonding than that possible for the rigid SU-8. Alternatively, the lower absorbance of 1002F compared to that of SU-8 near 532 nm may result in the conversion of a smaller fraction of the laser light into mechanical release energy and subsequent pallet detachment.

Conclusion

The use of 1002F resin combined with triarylsulfonium hexafluoroantimonate salts as a photoresist was characterized and its applicability to the fabrication of arrays of pallets demonstrated. The fluorescence of the new 1002F photoresist was significantly less than that of SU-8 enabling improved fluorescence sensitivity when 1002F was used as a substrate. Most of the residual fluorescence of 1002F structures is likely due to the fluorescence of the triarylsulfonium hexafluoroantimonate salts (or their byproducts). Thus, further reductions in the fluorescence await the development of photoacid generators with decreased fluorescence in the visible wavelengths. The aspect ratio of structures fabricated from 1002F resin was 4:1 under these conditions and exceeds that needed for the fabrication of the micropallet arrays for cell sorting. These preliminary results also suggest that 1002F is biocompatible in that cells grown on native or coated 1002F demonstrated excellent adhesion and metabolic behavior. Both the ability of the 1002F pallet arrays to support virtual walls as well as the low laser energies needed to detach 1002F pallets from a glass substrate suggest that 1002F is an excellent pallet substrate for the pallet array system. Moreover, 1002F is similar to SU-8 in chemical structure, therefore, 1002F can also be considered for applications currently using SU-8, such as DNA or protein immobilization to improve the signal-to-noise for fluorescence measurements. In addition to bioanalytical applications, the properties of 1002F will enable this photoresist to serve as an excellent substrate for a variety other BioMEMS, for example, cantilevers, fluidic channels, reaction chambers, fluid dispensers, and cell culture reservoirs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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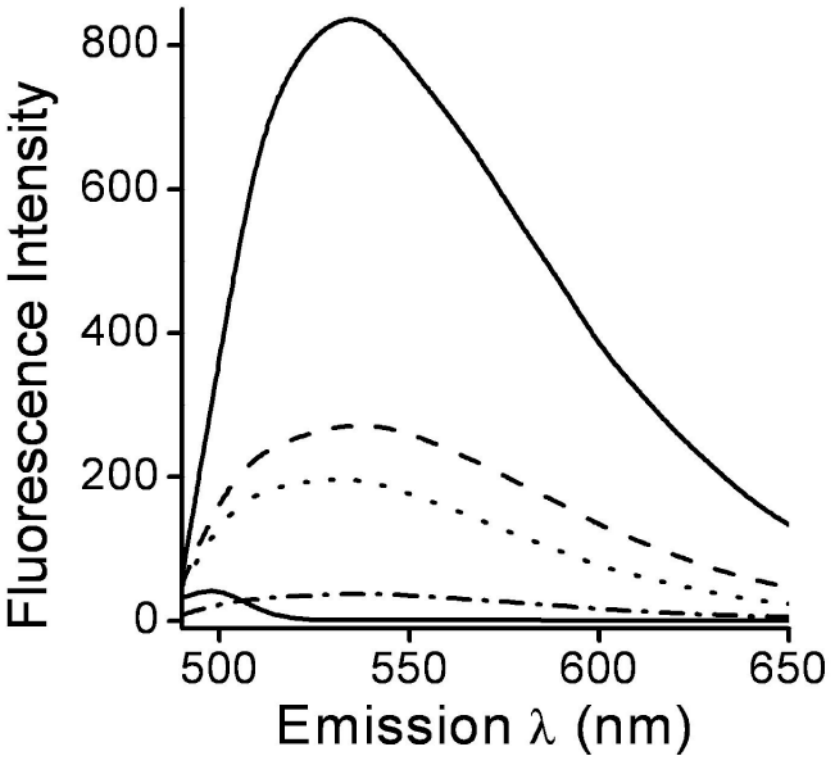

Figure 1.
Fluorescence of blocks of SU-8 and 1002F resin and photoresist. The blocks were excited at 485 nm with a ±2 nm band pass and the fluorescence measured from 490 to 650 nm with a ±2 nm band pass. The upper solid and dashed lines are SU-8 photoresist and SU-8 resin, respectively. The dotted and dash-dotted line are 1002F photoresist and 1002F resin, respectively. The lower solid line is PDMS. For technical reasons, the photoresist blocks were not treated with UV exposure and post-exposure baking.

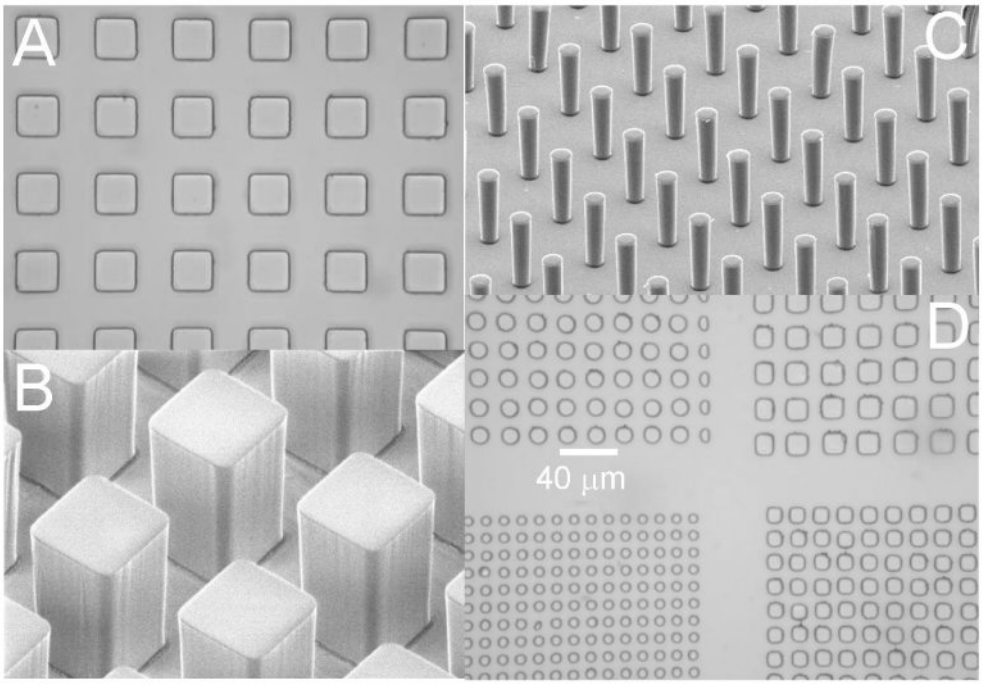

Figure 2.

Images of 1002F pallets. (A) Transmitted light microscopy of an array of pallets. Each pallet was square in cross section with a 50 μm side and 75 μm height. The distance between each pallet was 50 μm. (B) SEM of the pallets shown in (A). (C) SEM of an array of pallets. Each pallet was circular in cross-section with a 25 μm diameter and 100 μm height. (D) Transmitted light microscopy of four pallet arrays fabricated on a single glass slide. The pallets on the left side of the image were fabricated from a mask with circular openings of diameter 10 μm (top array) or 5 μm (bottom array). The pallets on the right side of the image were square openings with a side of 10 μm (top array) or 5 μm (bottom array). The spacing between the 5-μm and 10-μm pallets was 5 μm and 10 μm, respectively.

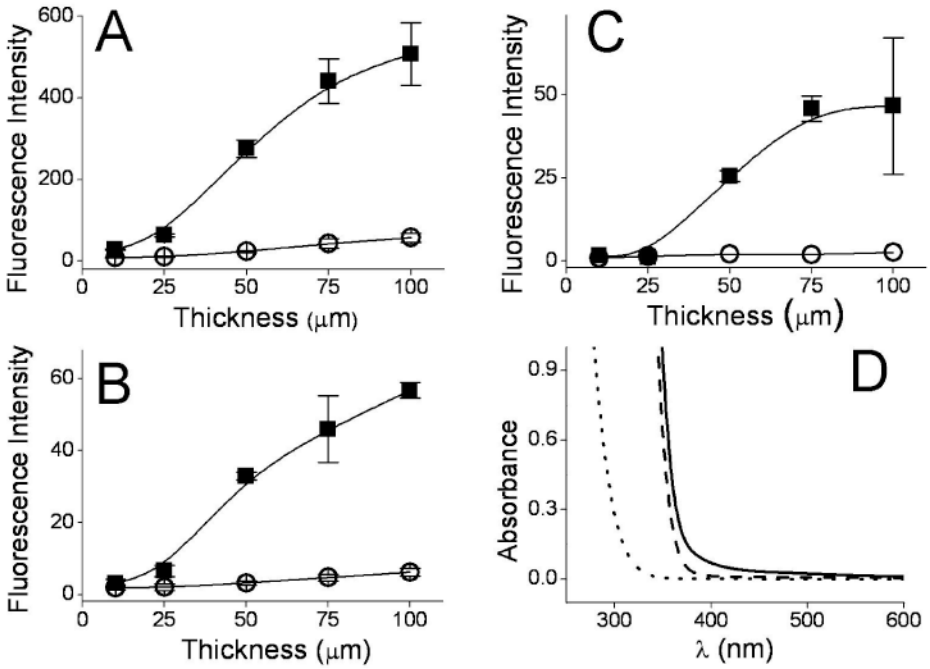

Figure 3.

Spectral properties of SU-8 and 1002F photoresists after UV exposure and post-exposure bake. (A) Fluorescence of SU-8 (solid squares) and 1002F (open circles) of lines (100 μm wide) of varying heights. The fluorescence was collected with a filter set designed for fluorescein (excitation filter 450-490 nm, dichroic 500 nm long pass, emission 520 nm long pass). (B,C) Same as (A) but the fluorescence was collected using a filter set for tetramethyl rhodamine (excitation filter 528-553 nm dichroic 565 nm long pass, emission 590-650 nm) (B) or Cy5 (excitation filter 590-650 nm, dichroic 660 nm long pass, emission 665-740 nm) (C). The error bars represent the standard deviation of 5 measurements. (D) The absorbance of glass (dotted line), 1002F photoresist (dashed line), and SU-8 photoresist (solid line) is shown at varying wavelengths.

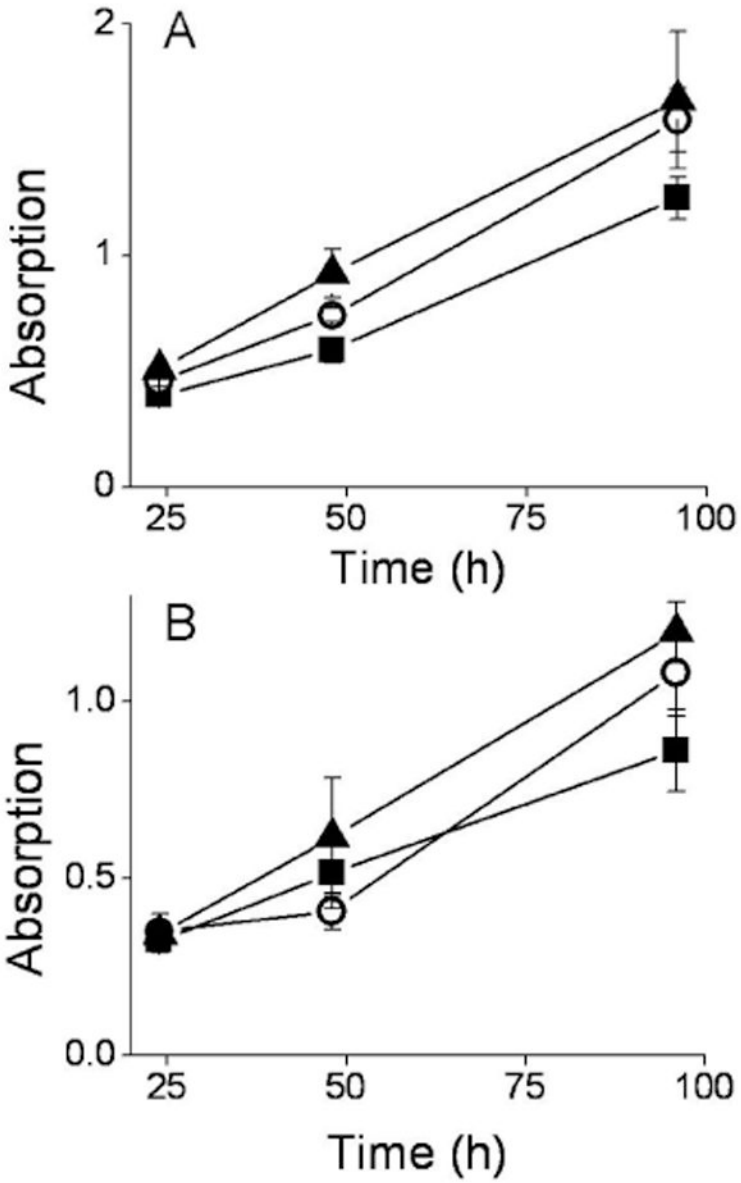

Figure 4.

Measurement of metabolism by XTT assay of cells grown on uncoated photoresists. RBL (A) or A172 (B) cells were cultured on glass (solid triangles), 1002F photoresists (open circles), or SU-8 photoresist (solid squares) for varying times. Shown on the "y" axis is the absorbance of the orange formazon product produced by metabolically active cells. The error bars represent the standard deviation of 4 measurements.

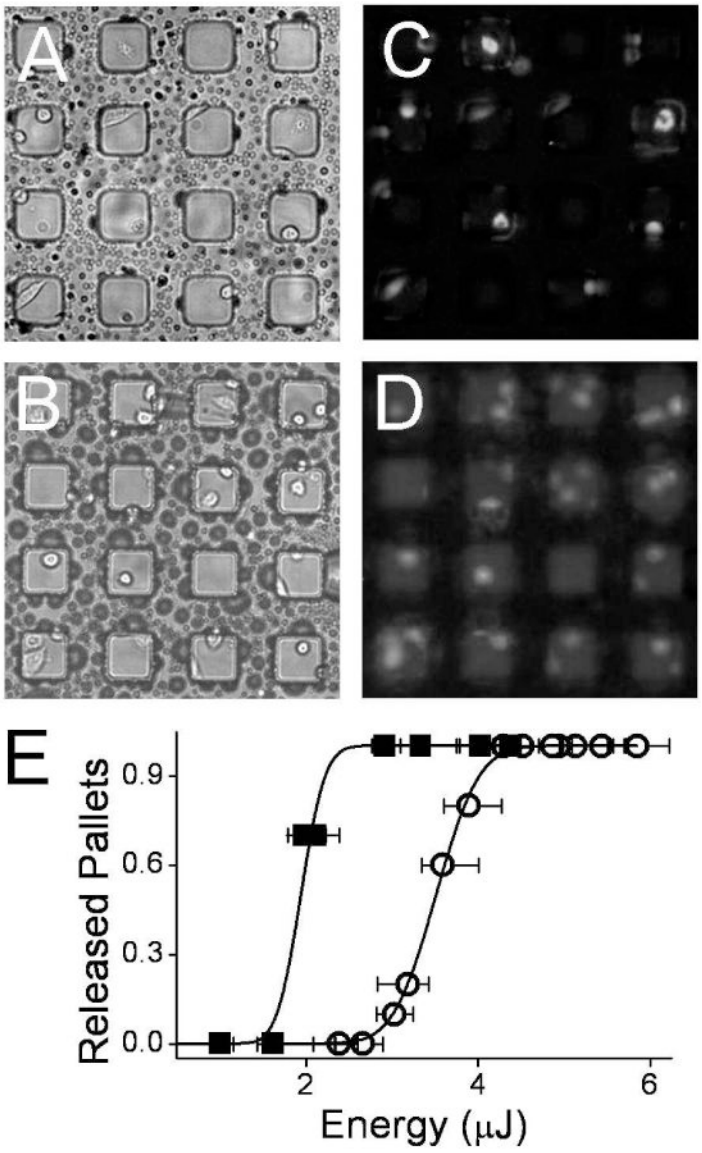

Figure 5.

Properties of SU-8 and 1002F pallets fabricated in arrays. (A,B) Transmitted light images of HeLa cells grown on 1002F (A) or SU-8 (B) pallets on arrays with virtual walls. (C,D) Fluorescence images of HeLa cells loaded with Oregon Green on 1002F (C) or SU-8 (D) pallet arrays. (E) Measurement of the threshold energy for pallet release. Shown on the "y" axis is the fraction of pallets released at each energy tested. Ten pallets were released for each data point. The energy of each pulse sent from the laser was measured. The "x" coordinate represents the average energy at a given laser setting and the error bars represent the standard deviation. The dimensions of the pallets in (A)-(E) were 50 μm (side) and 100 μm (height). The distance between each pallet was 50 μm.

Table 1

Process Parameters for 1002F Photoresist

Photoresist Thickness	1002F-10		1002F-50		
	10 μm	25 μm	50 μm	75 μm	100 μm
Spin coating Step 1 (rpm)	500	500	500	500	500
Spin coating Step 2 (rpm)	2000	1000	2200	1550	1200
Soft Bake Step 1 65°C (min)	10	10	20	25	30
Soft Bake Step 2 95°C (min)	10	20	40	50	60
UV exposure (mJ/cm^2)	480	660	1260	1920	2520
Post Exposure Bake Step 1 65°C (min)	1	1	1	1	1
Post Exposure Bake Step 2 95°C (min)	2	3	5	8	10
Develop (min)	2	4	6	8	12