SU-8 2000 rendered cytocompatible for neuronal bioMEMS applications

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Abstract: Microfabrication advances have resulted in small, cheap, and precise devices for biological microelectromechanical systems (bioMEMS). SU-8/SU-8 2000 is an attractive material for these applications because of its high-aspect ratio fabrication capability, dielectric properties, and thermochemical stability. Despite these advantages, the potential toxicity of SU-8 2000 may limit its use in cell-based applications. We show that <10% of primary neurons survived when cultured adjacent to or on top of untreated SU-8 2000. We evaluated the efficacy of various detoxification and surface treatments for SU-8 2000 in neuronal cultures after 7–21 days *in vitro*. Viability was improved to 45.8% ± 4.5% (mean ± standard error of the mean) following 3-day heat treatment (150°C) under vacuum, while UV exposure and CO_2 supercritical extraction did not improve survival. Furthermore, parylene coating (25 μm), in combination with heat and sonication (in isopropanol) treatments effectively masked the SU-8 2000 and led to 86.4% ± 1.9% viability. Glow discharge (oxygen plasma) treatment rendered the SU-8 2000 surface more hydrophilic and improved neuronal viability, possibly through improved cell adhesion. No organic leachants were detected by mass spectrometry before or after heat treatment or after sonication. However, XPS analysis revealed the presence of potentially neurotoxic elements, fluorine and antimony. Strategies to improve the cytocompatibility of SU-8 2000 with primary neurons will allow longer culture times and have applications for cell-based microfabrication. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res 89A: 138–151, 2009

Key words: neurons; microfabrication; MEMS; process optimization; toxicity

INTRODUCTION

Microfabrication advances have resulted in small, cheap, and precise devices for biological microelectromechanical systems (bioMEMS).[1] SU-8, a negative, near-ultraviolet (UV) photoresist, enables the manufacturing of high-aspect ratio thick MEMS structures.[2–4] The original SU-8 formulation used gamma-butyrolactone as the solvent,[2,3] while the more recent SU-8 formulations, using cyclopentenone as a solvent, are referred to as SU-8 2000.[4] Exposed SU-8/SU-8 2000 resists are thermally and chemically stable due to their aromatic functionality and highly crosslinked matrix,[3–7] making them ideal for the fabrication of high-aspect ratio 3D microelectrode culture systems. A 3D microelectrode system made using SU-8/SU-8 2000 requires long-term cytocompatibility.[8,9]

Cytocompatibility studies of SU-8/SU-8 2000 to date have focused on the original SU-8 formulation using both neuronal and nonneuronal cells and tissues. In addition, many current systems in use do not incorporate an appreciable amount of SU-8 (e.g., 5 μm insulation layer in Ayanda Biosystem's multielectrode arrays (MEAs)),[10–15] drastically reducing the potential toxic leachants. Neural applications have included dissociated cultures, as well as slice cultures. For example, in a microfluidic device made partially out of SU-8, chick embryonic motoneurons were viable for up to 48 h.[8] In another study, while electrophysiological activity was detected in neuronal cultures for 45–60 days within SU-8 containing MEAs, no quantitative viability assessment was performed.[16] Neuro-

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blastoma cells cultured on thick SU-8 2000 structures showed poor cell adhesion.[17] Several other studies have used 5 μm insulation layer SU-8-containing structures to monitor electrophysiological activity in slice preparations, yet viability as a function of materials in the devices was not evaluated.[10–13,15]

Because of the paucity of toxicity-related information for thick SU-8 2000 coatings with neuronal cell types, investigations on biocompatibility of cured SU-8 using nonneuronal cell types may be relevant in determining conditions that contribute to cell toxicity. For example, SU-8 implanted within stainless steel cages in a subcutaneous rodent model had less biofouling and similar numbers of leukocytes accumulate compared to controls, suggesting that SU-8 is biocompatible,[9] but SU-8 has also been found to be thrombogenic.[18] SU-8 has been shown to be toxic to cultured mouse fibroblasts, yet yielded minimal negative responses when implanted in rabbit muscle.[6] Others have also observed that untreated SU-8 is incompatible with mammalian cells, yet following HNO_3 and ethanolamine treatments HeLa cells preferred hydrophilized SU-8 surfaces compared to nonhydrophilized SU-8 at least up to 4 days *in vitro* (DIV).[19] Therefore, the cytocompatibility of SU-8/SU-8 2000 has not yet been established, likely due to differential cell responses and culture environments, variations in fabrication, material processing, and/or exposure to surface area.

Cytocompatibility of the SU-8 2000 series[4] (MicroChem Corp., Newton, MA) warrants investigation since it offers several processing advantages over the original SU-8 formulation, including significantly improved wetting, faster drying, and clean edge bead removal without the need for an intermediate bake step. In this study we present data showing that thick untreated SU-8 2000 substrates ($\geq$100 μm), which are often associated with high-aspect ratio structures, are not compatible with primary neuronal culture. We postulate that the poor cytocompatibility of SU-8 2000 resulted from two sources: (1) toxic leaching from the SU-8 2000 components; and (2) poor sustained neuronal adhesion. This study is the first reported characterization of SU-8 2000 cytocompatibility for *in vitro* neurobiological applications and presents several preparatory treatments for improved compatibility with primary neurons, which may be applicable to improvement of both SU-8 and SU-8 2000 materials.

MATERIALS AND METHODS

Fabrication of SU-8 2000 substrates

An empirically derived modification of SU-8 2000 substrate processing, based on manufacturer's recommended procedure[20] was used to fabricate high-aspect ratio (20:1) thick micro structures (up to 700 μm thick).[21] Similar adapted protocols have been used previously, with pre- and postbaking times and UV exposures in a range similar to that used here for obtaining thick high-aspect ratio structures from SU-8 2000.[22–25] A 100 μm (or 300 μm) thick layer of SU-8 2000 (either SU-8 2025 or SU-8 2050) (MicroChem Corp.) was spin-coated at 500 rpm (CEE Model 100CB Spinner, Rolla, MO) on a glass substrate (Fig. 1) and prebaked (30 min for the 100 μm samples or 4 h for the 300 μm samples) at 95°C to remove the solvent from the resist layer. The SU-8 2000 substrates were subsequently exposed to UV light (1000 mJ for the 100 μm samples and 2000 mJ for 300 μm samples) through a checkerboard-patterned mask to initiate crosslinking of the exposed SU-8 2000 plateaus (OAI HYBRALIGN Series 500 system, Optical Associates, Mask Aligner, San Jose, CA). Following the UV exposure the SU-8 2000 substrates were postbaked on a hotplate at 95°C (30 min for the 100 μm samples or 40 min for the 300 μm samples), changing the linear oligomer to a crosslinked network structure. Both the pre- as well as postbaking followed the alternatively recommended ramp procedure for baking on hot plate by initially ramping the temperature from 65 to 95°C in 10 min. The SU-8 2000 substrate was then developed using PGMEA (propylene glycol monomethyl ether acetate, Rohm and Haas Electronic Materials, North Andover, MA), removing the uncrosslinked regions and resulting in SU-8 2000 plateaus separated by the checkerboard pattern.

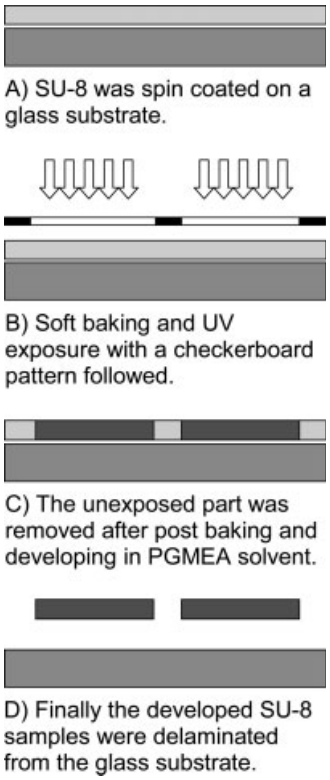

Figure 1. Microfabrication process for making SU-8 2000 samples. The different stages in the fabrication process include: spin coating, soft baking, UV exposure, post baking, developing in PGMEA, and finally delamination.

Individual 1 cm^2 × 100 μm (or 300 μm) thick SU-8 2000 plateau samples were separated manually using forceps. These were assayed as untreated samples and used for the various treatments described below.

SU-8 2000 treatments and coating

Initially, the effect of exposure to SU-8 2000 on neuronal culture viability was determined by culturing cells adjacent to untreated samples and reused SU-8 2000 samples (used previously in cell culture for 34 days, to test the effect of long-term exposure to cell culture media on toxicity). SU-8 samples were processed for reuse by rinsing with 70% ethanol, and sterile deionized water. After drying, substrates were recoated with poly-D-lysine. By placing cultures adjacent to the SU-8 2000 samples, we eliminated the variable of poor adhesion possibly contributing to the cell response. Next, the following treatments were employed to assess their ability to make SU-8 2000 more cytocompatible to neurons in culture adjacent to the SU-8 2000 samples: heating at 150°C for 3 days under vacuum (hard baking) (referred to as H hereafter), UV light exposure of 90 J/cm^2 (OAI HYBRALIGN Series 500 system, Optical Associates, Mask Aligner, San Jose, CA) (UV) and CO_2 supercritical extraction for 30 min (Autosamdri-815B, Tousimis Supercritical Dryer, Tousimis, Rockville, MD) (CO_2). The combination treatments tested were: UV + CO_2, H + UV, H + CO_2, H + UV + CO_2.

In addition, ultrasonication in isopropanol (Mettler Electronics model No. 4.6, 85 W) (S) of H treated SU-8 2000 samples was performed for 0, 1, 3, or 15 min, followed by oxygen plasma treatment (EMS-100, Electron Microscopy Sciences, Hatfield, PA) and assessed for cytocompatibility. Finally, the effect of 30, 90, 180, 270, or 360 s glow discharge oxygen plasma treatments (O_2) or parylene coating (25 μm) (parylene C, poly(monochloro-*p*-xylylene), Cookson Electronics, Providence, RI; PDS 2010 LABCOATER, Specialty Coating Systems, Indianapolis, IN) (P) on the cytocompatibility of H + S treated SU-8 2000 was also studied, to improve cell adhesion while testing for exposure to possible toxic leachants. The cultures tested in the experiments described here, therefore, were on top of the SU-8 2000 samples.

Tissue culture treated polystyrene surfaces (Corning, Corning, NY) or glass substrates (VWR Scientific, West Chester, PA) with or without a 25 μm parylene coat served as controls. All cell culture surfaces were coated with poly-D-lysine (100 μg/mL) before plating. Table I summarizes the experimental design for testing of the SU-8 2000 treatments. A subset of experiments was designated to simultaneously vary a number of conditions to identify a set of adequate conditions for further testing. Once treatment conditions suggested efficacy, a time series was evaluated with additional power obtained with group replication (e.g., Figs. 4 and 6).

Sterilization of SU-8 2000 samples

SU-8 2000 samples were attached to polystyrene cell culture wells and sterilized with 70% ethanol under a laminar flow hood with UV light for 30 min, followed by rinsing in sterile filtered deionized (DI) water under UV light for 30 min, aspirating off the DI water, and exposing to 60 min of continued UV. SU-8 2000 samples that were attached to glass were sterilized by 45 min of UV exposure only. During sterilization the samples were placed at distances ≥65 cm from the laminar flow hood UV lamp with an intensity ≤0.6 J/cm^2.

Neuronal cell culture

Timed-pregnant Sasco Sprague-Dawley rats (Charles River, Wilmington, MA) were anesthetized using halothane (Halocarbon Laboratories, River Edge, NJ) at embryonic day 17–18 and decapitated. Fetuses were removed and placed in Hanks balanced salt solution (HBSS, Invitrogen, Carlsbad, CA). All procedures involving animals were approved by the Institutional Animal Care and Use Committee (IACUC) of the Georgia Institute of Technology or that of the Southern Illinois University, following NIH guidelines for the care and use of laboratory animals (NIH Publication No. 85-23 Rev. 1985). Cerebral cortices and hippocampi were isolated and dissociated using prewarmed trypsin (0.25%) + 1 m*M* EDTA (Invitrogen) for 10 min at 37°C followed by deoxyribonuclease I (0.15 mg/mL, Sigma, St. Louis, MO) in HBSS (or papain 2 mg/mL in Hibernate-calcium) (BrainBits LLC, Springfield, IL). The tissue was triturated with a flame-narrowed Pasteur pipette and centrifuged at 1000 rpm for 3 min and the cells were resuspended in Neurobasal medium + 2% B-27 + 500 μ*M* L-glutamine (Invitrogen). Cultures were plated in standard polystyrene six-well plates (Corning) or 24 mm × 60 mm glass cover slips (VWR Scientific) both pretreated with 100 μg/mL poly-D-lysine (Sigma, St. Louis, MO). As summarized in Table I, neurons were plated either adjacent to or directly on test biomaterials (e.g., SU-8 2000, glass controls, parylene coated glass controls), which were adhered to polystyrene wells or glass using polydimethylsiloxane (PDMS, Sylgard® 184 and 186 Silicone Elastomer Kit, Dow Corning Corp., Midland, MI). Cortical cells were plated at a density of 1000 cells/mm^2; hippocampal cells were plated at 500 or 1000 cells/mm^2. These plating densities have been used previously for MEA studies and result in a cell density which permits recording at the electrodes while maintaining discernable cell–cell distances.[26,27] Cultures were incubated at 37°C and 5% CO_2-95% humidified air, and fed every 3–4 DIV by replacing half the media with media prewarmed to 37°C. Both embryonic cortical and hippocampal neurons were used to evaluate various SU-8 2000 treatments on two commonly used culture types, which allows the isolation of cells without causing irreversible damage of sheared axons and dendrites, as seen in adult tissue.[28–30] A culture time of 1 week was used to test acute effects of the SU-8 2000 (surface chemistry related) on the cultures that were plated on top of the SU-8 2000, while a 3 week culturing time point was used for assessing the chronic effects of SU-8 2000 leaching on cultures that were plated adjacent to the SU-8 2000. We used a sensitive and inexpensive *in vitro* testing method where the test material was directly exposed to the cell culture,[31] and immobilized so as not to disturb the cell culture by floating or scratching it.[32]

TABLE I
Summary of the Various SU-8 2000 Treatments Studied

Treatments	*n* Per Treatment	Cell Type	Plating Location with Respect to SU-8 2000	Plating Density (cells/mm^2)	No. of SU-8 2000 Samples per Culture, Sample Dimensions	Controls	Live/Dead Time Point (DIV)	Results Figure No.
New Reused	4 5	Cort.	Adjacent	1000	4, 10 × 10 × 0.1 mm^3	Polystyrene, $n = 12$	21	2
H UV CO_2 UV + CO_2 H + UV H + CO_2 H + UV + CO_2 Untreated	4	Cort.	Adjacent	1000	4, 10 × 10 × 0.1 mm^3	Polystyrene, $n = 4$	21	3
H H + S (1 min, 70% IPA) H + S (3 min, 70% IPA) H + S (15 min, 70% IPA)	2	Hipp.	On top	500	2, 10 × 10 × 0.3 mm^3	Glass slip, $n = 2$	7	4
H + S (30 min) H + S (30 min) + P (25 μm)	5	Cort.	On top	1000	1, 10 × 10 × 0.1 mm^3	25 μm Parylene coated glass slip, $n = 5$	7	5
H + S (1 min, 70% IPA) + O_2 (30, 90, 150 sec) H + S (1 min, 100% IPA) + O_2 (30 sec) H + O_2 (30 sec) H + S (1 min, 70% IPA) H	Eight fields across one culture	Hipp.	On top	1000	1, 10 × 10 × 0.3 mm$_3$	Glass slip, $n = 2$	7	6

The various bulk detoxification and surface treatments used are summarized. All culture surfaces were coated overnight with 100 μg/mL poly-D-lysine.
n, number of replicates; DIV, days *in vitro*; Cort., E17-18 primary cortical neurons; Hipp., E17-18 primary hippocampal neurons; New, untreated = new SU-8 2000 without any treatment mentioned in the table; reused, SU-8 2000 without any treatment mentioned in this table but previously used in cell culture for 34 days; H, heating at 150°C for 3 days in vacuum; UV, UV light exposure of 90 J/cm^2; CO_2, CO_2 supercritical extraction for 30 min; S, isopropanol sonication; P, parylene coating; O_2, O_2 plasma treatment.

Assessment of cell viability for SU-8 2000 treatments

Cell viability was measured using fluorescent probes for distinguishing live and dead cells (LIVE/DEAD Viability/Cytotoxicity Kit; Molecular Probes, Eugene, OR) after 7 or 21 DIV. Cell cultures were incubated with 2 μ*M* calcein AM (live cell probe) and 4 μ*M* ethidium homodimer-1 (EthD-1) (dead cell probe) at 37°C for 30 min and rinsed in 0.1*M* Dulbecco's phosphate buffered saline (D-PBS, Invitrogen) before imaging. This methodology does not take into account cells that have been washed away from the surface during media changes and rinses. Comparisons between plating and final densities of cells on the different surfaces, therefore, provide an estimate of the number of cells lost, assuming no proliferation.

Data collection and statistical analysis

Cell viability was assessed using fluorescent microscopy performed with either a Nikon Eclipse TE300 (Nikon Instruments, Melville, NY), an Olympus BX51WI (Melville, NY), or a Zeiss 510 Laser Scanning Confocal Microscope (Carl Zeiss AG, Göttingen/Germany). Two to eight photomicrographs were taken per cell culture well for quantification. Cell culture data are presented as % viability and live cell density. Percent cell viability is the ratio of live over total adherent cells (live + dead). Live cell density or live cells/mm^2 is the live cell count per area of substrate. Percent of viable cells (which is sensitive to the dead cells count) was used for the adjacent cell culture experiments to assess cell death as a result of toxic leaching, whereas the live cell density was used for cultures placed on top of the sample, as the viability of cells that remained attached to the test biomaterial surfaces is of interest for material characterization (Table I). Data are presented as mean ± standard error of the mean. General linear model ANOVA was performed followed by Student-Newman-Keuls pairwise comparisons ($p < 0.05$ was considered significant).

Mass spectrometry, X-ray photoelectron spectroscopy, and contact angle measurements

Mass spectrometry analysis was conducted to identify possible organic leachants from SU-8 2000 that could be toxic to the cells. Glass vials were rinsed with ethanol, DI water, and isopropanol and filled with ACS reagent grade isopropanol (2 mL). In one of the vials five SU-8 2000 samples (1cm × 1 cm × ~100 μm) were added (S), while in another five SU-8 2000 samples from the same batch that were heat treated in a vacuum oven at 150°C for 3 days were added (H + S) while the third vial was used as a blank isopropanol control (Blank S). Vials were placed in an ultrasonication bath for 30 min, followed by removal of the solvent phase for analysis by mass spectrometry. Gas chromatography (J & W Column, DB-5, 30 m length, 0.25 mm I.D.; J & W Scientific, Folsom, CA) was used to separate the potential leachants in the isopropanol solvent, followed by electron ionization mass spectrometry (VG 70SE, VG Instruments, Manchester, UK). Likewise, liquid chromatography (Agilent 1100 Series, Supelco C18, 100 mm length, 1 mm I.D.; Agilent Technologies, Palo Alto, CA) was used to separate the potential leachants, followed by electrospray mass spectrometry (Micromass Quattro LC, Manchester, UK). Separate samples were concentrated until completely dry, and reconstituted in 100 μL of isopropanol. Samples were injected onto a gas chromatography column followed by electron ionization mass spectrometry, as well as vaporized in a heated solids probe followed by electron ionization mass spectrometry, or reconstituted in 200 μL of water and subjected to liquid chromatography followed by electrospray mass spectrometry.

The elemental composition analyses of the SU-8 2000 surfaces after various bulk detoxification and surface treatments were performed using X-ray photoelectron spectroscopy (XPS) analysis (Surface Sciences Instruments Model SSX-100 Small Spot ESCA Spectrometer equipped with a monochromatized Al K-alpha X-ray source (1486.6 eV); Mountain View, CA). The operating pressure in the analysis chamber was maintained at 3.0×10^{-9} Torr or lower during the measurements. Each sample was scanned at 1–2 spots. For each spot, general survey scans (analyzer pass energy of 150 eV) were obtained for a sample from each of the above treatments followed by high resolution C1s and O1s scans (analyzer pass energy of 50 eV). Spectra were collected at a 100 μm diameter X-ray spot size. The binding energies (BEs) were referenced to the C1s (C-H) peak at 285.0 eV, to compensate for the effects of charging. All treatments were done as described previously, except the oxygen plasma treatment which was conducted in a Plasma Therm RIE 700 series reactive ion etcher (Plasma-Therm, Saint Petersburg, FL) at a pressure of 200 mTorr, O_2 flow of 100 standard cubic centimeters per min O_2, 200 W power for 1 min.

The effect of oxygen plasma treatment on SU-8 2000 surface hydrophilicity was assessed by measuring contact angle. The ambient air-water-substrate contact angle measurements (2 μL of DI H_2O) were taken with a Rame-Hart 100-00 goniometer (Mountain Lakes, NJ) fitted with a digital camera and analyzed using in-house image analysis software. Table II summarizes the various analytical techniques used to study the different SU-8 2000 treatments.

RESULTS

Assessment of cell viability resulting from exposure to fresh and reused SU-8 2000

At 21 DIV cortical neuronal cultures adjacent to SU-8 2000 samples had significantly lower viability than the plain polystyrene control groups (6.6% ± 6.6% vs. 70.9% ± 3.7%) (Fig. 2), indicating that neurons die when cultured in a media source contacting SU-8 2000. Soaking SU-8 2000 substrates in culture medium at 37°C during cortical neuronal culture improved neuronal survival in subsequent cultures; neuronal viability was improved (to 40.8% ± 5.5%) in cultures growing adjacent to reused SU-8 2000, suggesting that toxic leachants may be removed from SU-8 2000 during prior cell culture period.

TABLE II
Summary of the Analytical Techniques Used for Assessing SU-8 2000 After Various Treatments

Analytical Techniques	Treatments
Mass spectrometry 1. GC + EIMS 2. LC + ESMS 3. Samples dried and reconstituted in 100 μL isopropanol + GC + EIMS 4. Samples dried and reconstituted in 100 μL isopropanol + HPEIMS 5. Samples dried and reconstituted in 200 μL water + LC + ESMS	Blank S S H + S
X-ray photoelectron spectroscopy	Untreated H S H + S H + S + O_2 H + S + P H + S + P + O_2
Contact angle goniometry	Untreated O_2

Mass spectrometry, X-ray photoelectron spectroscopy, and contact angle goniometry techniques were used for assessing SU-8 2000 after the various treatments.

GC, gas chromatography; EIMS, electron ionization mass spectrometry; LC, liquid chromatography; ESMS, electro spray mass spectrometry; HPEIMS, heated probe electron ionization mass spectrometry; Blank S, 30 min blank isopropanol sonicated control; Untreated, new SU-8 2000 without any treatment; H, heating at 150°C for 3 days in vacuum; S, sonication in isopropanol for 30 min; O_2, O_2 plasma treatment for 1 min; P, parylene coating of 25 μm.

Assessment of different bulk detoxification treatments for SU-8 2000

At 21 DIV, with the exception of the heat treatment (H), all other treatments (UV, CO_2, UV + CO_2, H + UV, H + CO_2, H + UV + CO_2) resulted in neuronal viability in adjacent cultures significantly lower than control cultures (Fig. 3). The heat treatment resulted in viability of 45.8% ± 4.5% as compared to 61.1% ± 5% in polystyrene controls. Neuronal viability following UV exposure (0.3% ± 0.3%) and CO_2 supercritical extraction (0.1% ± 0.15%) treatments was significantly less than untreated SU-8 2000 samples (6.6% ± 6.6%). The measured cell densities for treatments and control groups at the end of the culturing period were lower then the cell densities at plating (500–1000 cells/mm^2) indicating cell loss after plating. The control viabilities in the range of 60–70% at the time point tested are consistent with a previous report.[33] Comparisons between plating and final densities of cells on the different surfaces provide an estimate of the loss of cells from these materials, assuming no proliferation.

Heat plus ultrasonication in isopropanol for 15 min resulted in an increased density of live cells on SU-8 2000 beyond that obtained through heat treatment alone (Fig. 4). The plating of cells in this experiment was on top of the SU-8 2000 samples, indicating that sonication over a period of time improves adhesion of the neurons to SU-8 2000 with consequent better survival, but with less effect on the ratio of live to total cells.

Assessment of adhesive surface treatments for SU-8 2000

There was a significant improvement in live cell density (for cells cultured on top of the SU-8 2000 samples) on heat and sonication treated SU-8 2000 substrates following parylene coating (25 μm) (556.9 ± 68.3 live cells/mm^2) compared to uncoated (308.6 ± 81.1 live cells/mm^2), which was comparable with parylene-coated glass controls (626.2 ± 58.2 live cells/mm^2) (Fig. 5). Oxygen plasma treatment improved live cell density and greatly increased neurite outgrowth for cells cultured on top of the SU-8 2000 samples (Fig. 6). Figure 6 also confirms the relative benefit of the heat + sonication (H + S) treatments reported in Figure 4.

Mass spectrometry, X-ray photoelectron spectroscopy, and contact angle measurements

Mass spectrometry analyses of all the different treatments did not indicate the presence of organic-based leachants from the SU-8 2000. The chromatograms and mass spectrograms for each of the treatments were indistinguishable from controls for all treatments (data not shown).

In the absence of any surface treatment such as parylene coating or oxygen plasma treatment, XPS analysis showed that the SU-8 2000 bulk detoxification treatments do not change the oxygen to carbon (main elemental components of SU-8 2000) ratio in SU-8 2000, which was ~0.2. However, XPS analysis revealed the presence of fluorine. Analysis showed that after oxygen plasma treatment, the oxygen to carbon ratio of SU-8 2000 increased from ~0.2 to ~1.4, and fluorine and antimony were also detected. Parylene C coating on the SU-8 2000 resulted in an XPS spectra without oxygen peaks, but chlorine peaks were evident, as is characteristic of the parylene C molecular structure.[34] After oxygen plasma treatment of the parylene coated SU-8 2000 however, the oxygen to carbon ratio increased from 0.0 to ~0.4. The presence of silicon in the SU-8 2000 is attributed to contamination from the processing environment, such as previous materials used in the processing equipments, since it is not normally expected to be present in SU-8/SU-8 2000.[2,3,4,35,36] Table III summarizes the XPS results.

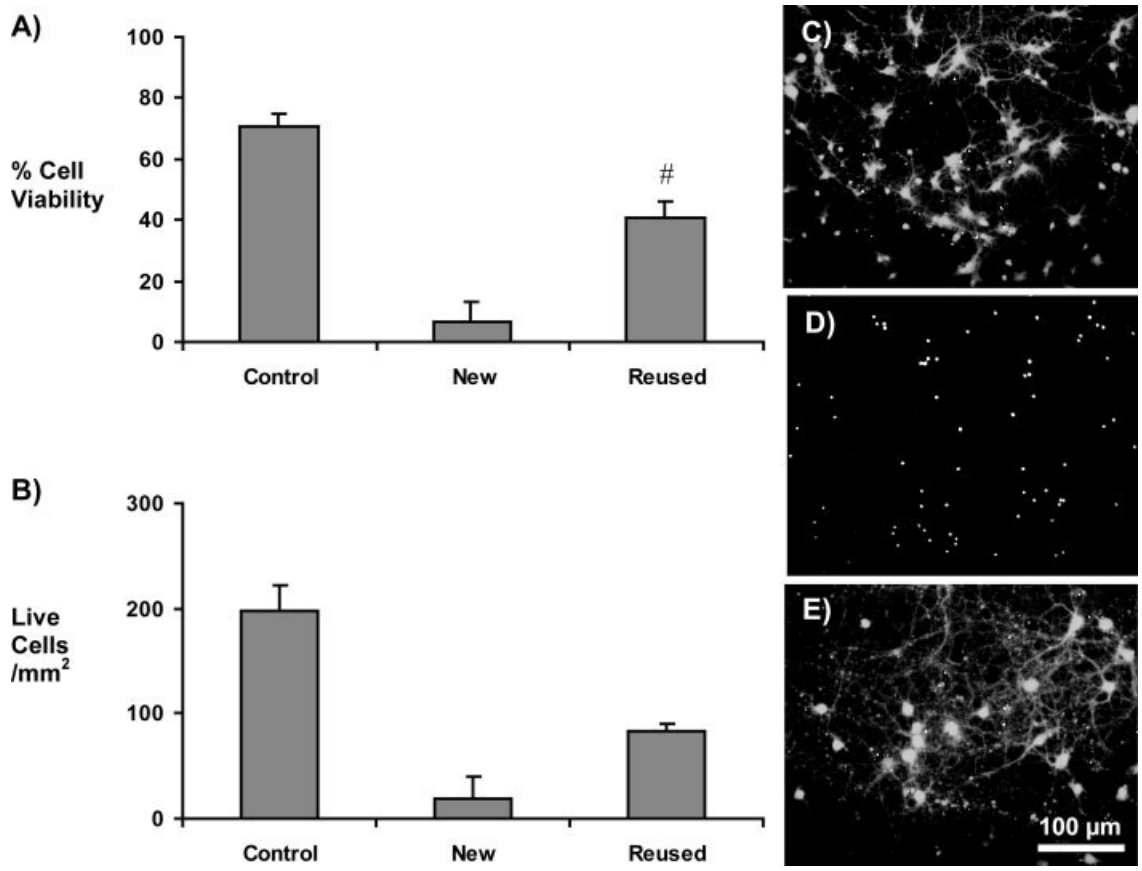

Figure 2. Assessment of cell viability of neural cultures plated adjacent to fresh and reused SU-8 2000 samples. Cellular response to SU-8 2000 exposure quantified as % cell viability (A) and live cells/mm^2 (B), with representative photomicrographs of neuronal cultures plated on polystyrene controls (Control, C), on polystyrene adjacent to new SU-8 2000 samples (New, D), and on polystyrene adjacent to reused SU-8 2000 samples (Reused, E) labeling live cells (gray) and dead cell nuclei (white). Neural cultures adjacent to SU-8 2000 samples that have been used previously in cell culture (Reused) have significantly higher viability than parallel cultures plated adjacent to new SU-8 2000 samples (New) (#$p < 0.005$).

Contact angle measurements were performed to study the effect of oxygen plasma treatments on the SU-8 2000 surface hydrophobic–hydrophilic character. The water contact angle was reduced from 72.9° ± 1.5° to 37.0° ± 2.3° after oxygen plasma treatment.

Thus, while mass spectrometry reduced the possibility of organic leachants from the SU-8 2000 after the heat treatment and during the isopropanol sonication, XPS analysis pointed to fluorine and antimony as candidate leachants from the SU-8 2000. Parylene coating resulted in the SU-8 2000 surface being masked of fluorine and antimony and any other potential leachants, while oxygen plasma treatment increased the oxygen groups on the SU-8 2000 surface, thereby making it more hydrophilic, as evidenced by the decreasing water contact angle measurements. While oxygen plasma appeared to expose low levels of fluorine (from a few nanometers below the SU-8 surface), the fluorine levels are only 10% of those detected after extraction by sonication alone (presumably being washed away during the poly-D-lysine coating prior to cell culture). This suggests that oxygen plasma treatment (which slightly etches the surface) after heat and sonication treatments, simply exposes the left-over fluorine and antimony from depths below the SU-8 surface (which may not be sensed by the cells), in addition to removing any that was accessible to XPS after the sonication treatment alone.

DISCUSSION

We found that SU-8 2000 is not cytocompatible to primary cortical or hippocampal neuronal cultures. While mass spectrometric analysis did not detect any organic leachants from the SU-8 2000, XPS analysis pointed to fluorine and antimony as candidate leachants from the SU-8 2000. The idea of toxic leaching is supported by poor viability of neuronal cultures plated adjacent to the SU-8 2000 samples. Both fluorine and antimony have the potential to be toxic and are present in the photo acid generator within the SU-8 2000 formulation.[2,35–38] Heat treat-

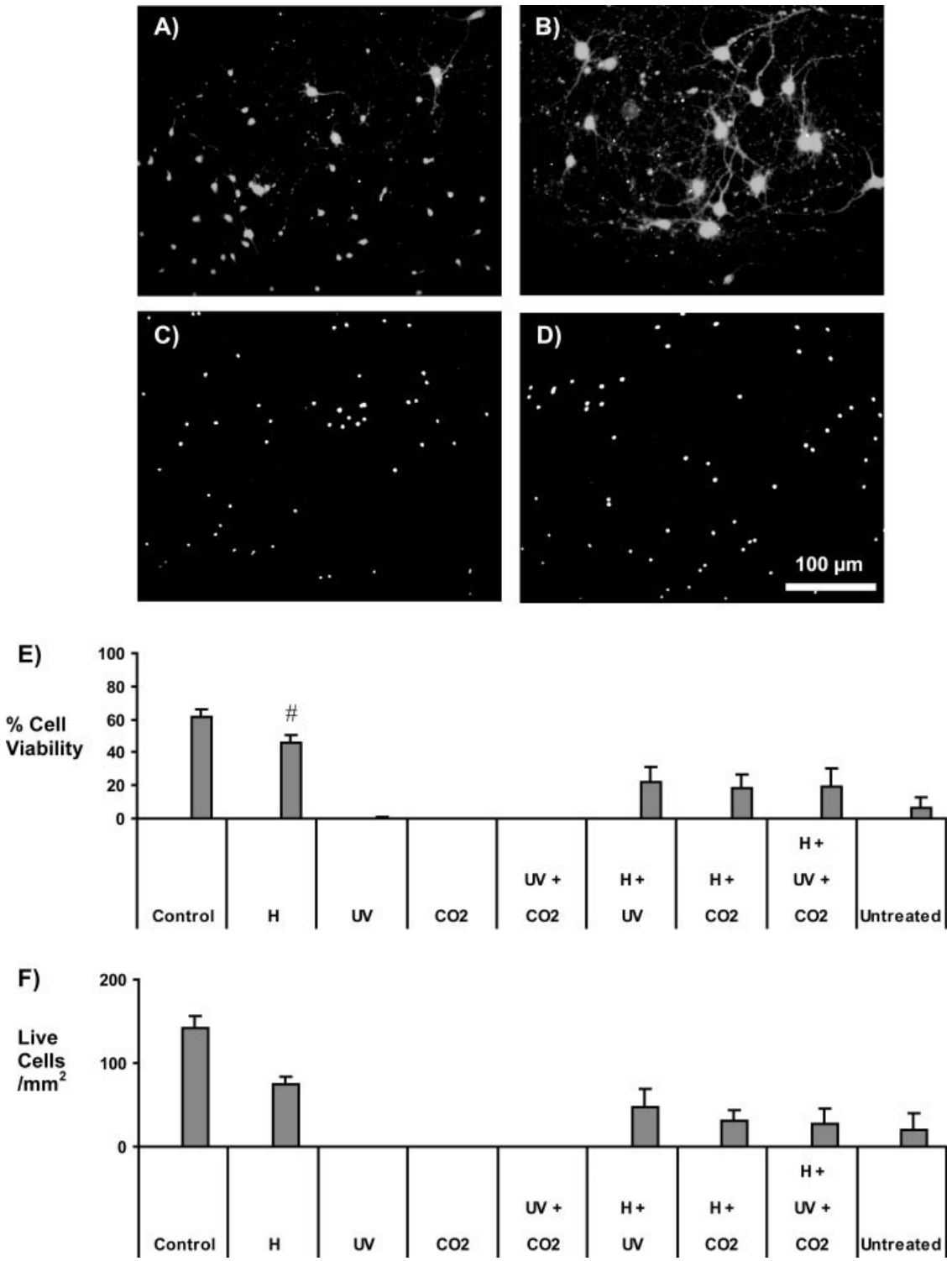

Figure 3. Assessment of different bulk treatments on SU-8 2000 samples for improving cytocompatibility with adjacent neuronal cultures. Representative photomicrographs of neuronal cultures plated on polystyrene controls (Control, A), on polystyrene adjacent to SU-8 2000 samples that were heat treated (H, B), on polystyrene adjacent to SU-8 2000 samples that were treated with UV exposure (UV, C), on polystyrene adjacent to SU-8 2000 samples that were treated with CO_2 under supercritical conditions (CO_2, D) labeling live cells (gray) and dead cell nuclei (white). The cellular response to these treatments and their combinations are quantified as % cell viability (E), and live cells/mm^2 (F). Cell viability following heat treatment (H) was significantly improved compared to the other detoxifying treatments ($^{\#}p < 0.05$).

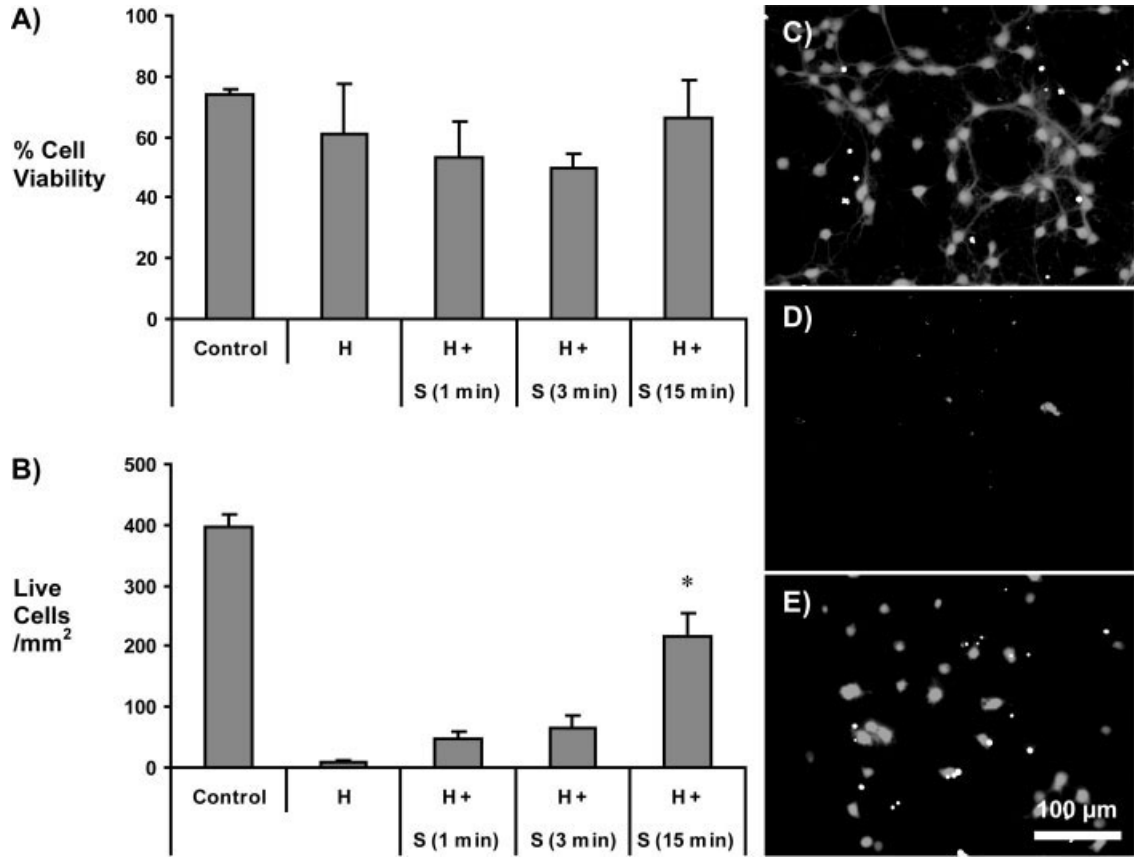

Figure 4. Assessment of sonication of SU-8 2000 in isopropanol for improving cytocompatibility with neuronal cultures. Neuronal cultures plated on top of SU-8 2000 after heat plus sonication treatments quantified as % cell viability (A) and live cells/mm^2 (B), with representative photomicrographs of neuronal cultures plated on glass controls (Control, C), on heat treated SU-8 2000 samples (H, D), on heat treated SU-8 2000 samples with 15 min of sonication in isopropanol (H + S (15 min), E) labeling live cells (gray) and dead cell nuclei (white). Longer periods of sonication in isopropanol produced better live cell density in culture. Live cell density for the 15 min isopropanol sonication treatment in addition to the heat treatment (H + S (15 min)), was significantly higher than the live cell density following the other detoxifying treatments (*$p < 0.0005$).

ment, isopropanol sonication, parylene coating, and oxygen plasma treatment improved SU-8 2000 cytocompatibility. Together these treatments improved viability to equivalent levels of control grade glass or parylene coated glass, thus providing protocols to render SU-8 2000 more cytocompatible for biological applications with neurons.

When cells were cultured adjacent to (but not touching) SU-8 2000, they were exposed constantly to the SU-8 2000 through the cell culture media, a similar scenario to many BioMEMs applications. Decreased viability for cultures plated adjacent to SU-8 2000 samples indicated that SU-8 2000 contained one or more extractable substances toxic to neurons, in agreement with previous findings.[6] This tested the effect of gradual leaching that may occur from the SU-8 2000 into the cell culture media. Reusing the SU-8 2000 over two rounds of cell culture possibly aided in the slow removal of these toxic agents from the SU-8 2000 via passive diffusion, thereby rendering it more cytocompatible. This indicated a possibility to detoxify SU-8 2000 through diffusive removal of leachants from the SU-8 2000. While we cannot rule out that protein adsorption at the surface of the SU-8-2000 occurred over the longer incubation periods, it is not likely to be substantial enough to prevent the diffusion of smaller molecular species such as fluorine and antimony, since the adsorbed protein is believed to not form more than a monolayer over the surface.[39] Furthermore, the poly-D-lysine may have formed a monolayer over the adsorbed protein layer, normalizing the effect of the protein layer on the cell culture. This is supported by the fact that the % cell viabilities for new and reused controls were statistically indistinguishable from each other (data not shown). Control experiments demonstrated that the adhesive PDMS used to attach the SU-8 2000 to the culture dishes had no adverse effects on cytocompatibility (data not shown).

XPS analysis revealed the presence of fluorine on the SU-8 2000 after the sonication treatment alone

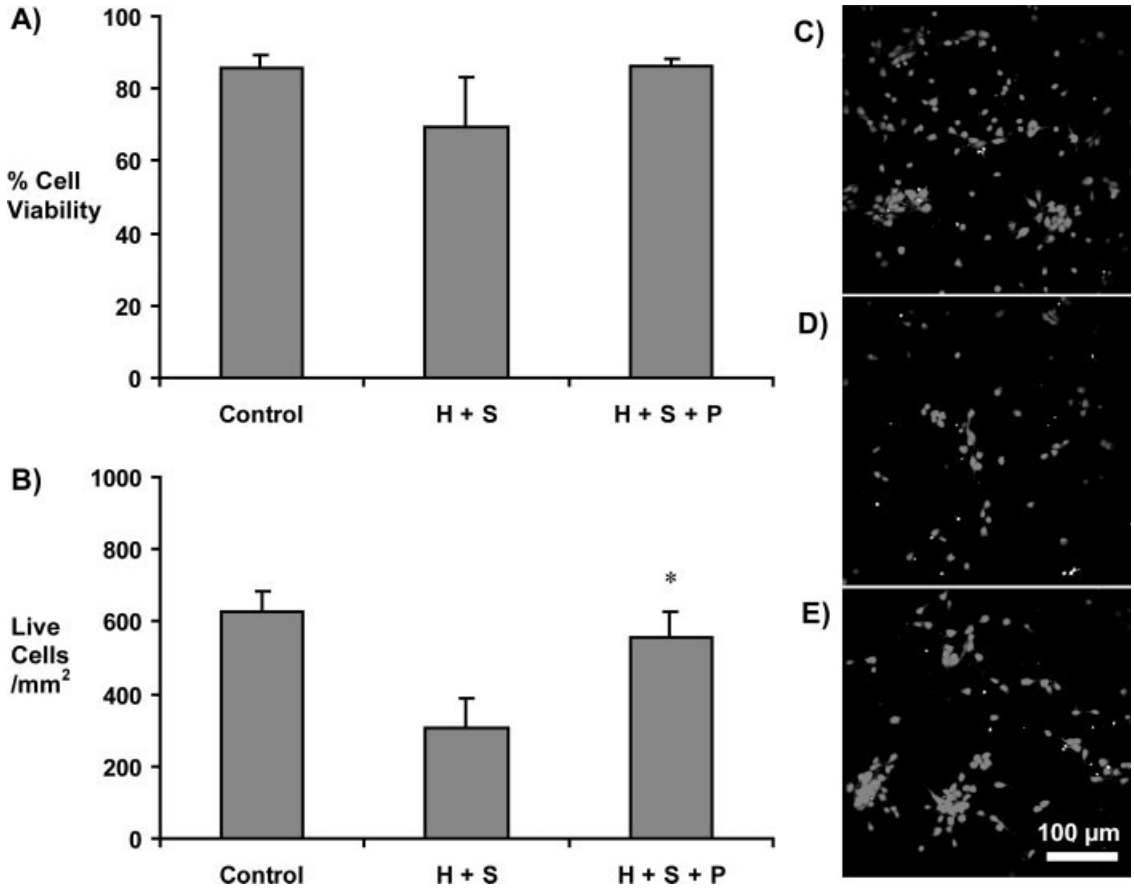

Figure 5. Assessment of parylene coating of SU-8 2000 samples for improving cytocompatibility with neuronal cultures. Neurons were plated on top of SU-8 2000 samples treated for 30 min by sonication in isopropanol following the heat treatment with or without parylene coating. Cellular responses are presented as % cell viability (A) and live cells/mm^2 (B), along with representative photomicrographs of neural cultures plated on parylene coated glass controls (Control, C), on heat treated plus isopropanol sonicated SU-8 2000 samples (H + S, D), on H + S treated SU-8 2000 samples followed by parlyene coating (H + S + P, E) labeling live cells (gray) and dead cell nuclei (white). Heat plus sonicated SU-8 2000 with parylene coating (H + S + P) resulted in significantly improved live cell density compared to just the heat plus sonicated SU-8 2000 samples (H + S), and was statistically indistinguishable from the control cultures (Control) (*$p < 0.05$).

(20%), indicating that the sonication treatment assisted in extracting fluorine from at least a shallow depth in the SU-8 2000 substrate. Furthermore, minimal fluorine was detected after the heat treatment alone (~1%), indicating possible diffusive removal aided by heat. Finally, after heat followed by sonication treatment, no fluorine was detected, indicating that this treatment combination resulted in the removal of fluorine from at least a shallow depth into the SU-8 2000 surface. This also explains the observation that after 1 min of oxygen plasma treatment, which etches the SU-8 2000 surface by a few nanometers, low amounts of fluorine as well as antimony were detected, correlating with other studies.[40] While fluorine and antimony from the photo acid generator are potentially toxic to the cells,[2,35–38] cyclopentenone (the solvent used in the SU-8 2000 formulation, boiling point 130°C), and PGMEA (solvent used in developing SU-8 2000, boiling point 146°C) may also be toxic to neurons.[4,41,42] The heat and oxygen plasma treatments may remove residual levels of these solvents through faster diffusion and oxidation, respectively.

Sustained heating of SU-8 2000 for 3 days at 150°C represents hard-baking of the material, which may enhance neuronal viability by three possible mechanisms: (1) by allowing for the crosslinking process of SU-8 2000 to reach completion as the photoacid actively crosslinks the resist using thermal energy, effectively decreasing the void volume inside the polymer, increasing its viscosity, and thereby reducing the diffusion rates of the photocatalyzing acids as well as other species in the system, such as solvent molecules[3,4]; (2) by the faster diffusion of the toxic leachants out of the SU-8 2000 under high temperature and vacuum conditions; and (3) by making changes in the surface characteristics of the SU-8 2000. The mechanical properties of cured SU-8 films differ with the baking temperature and this differ-

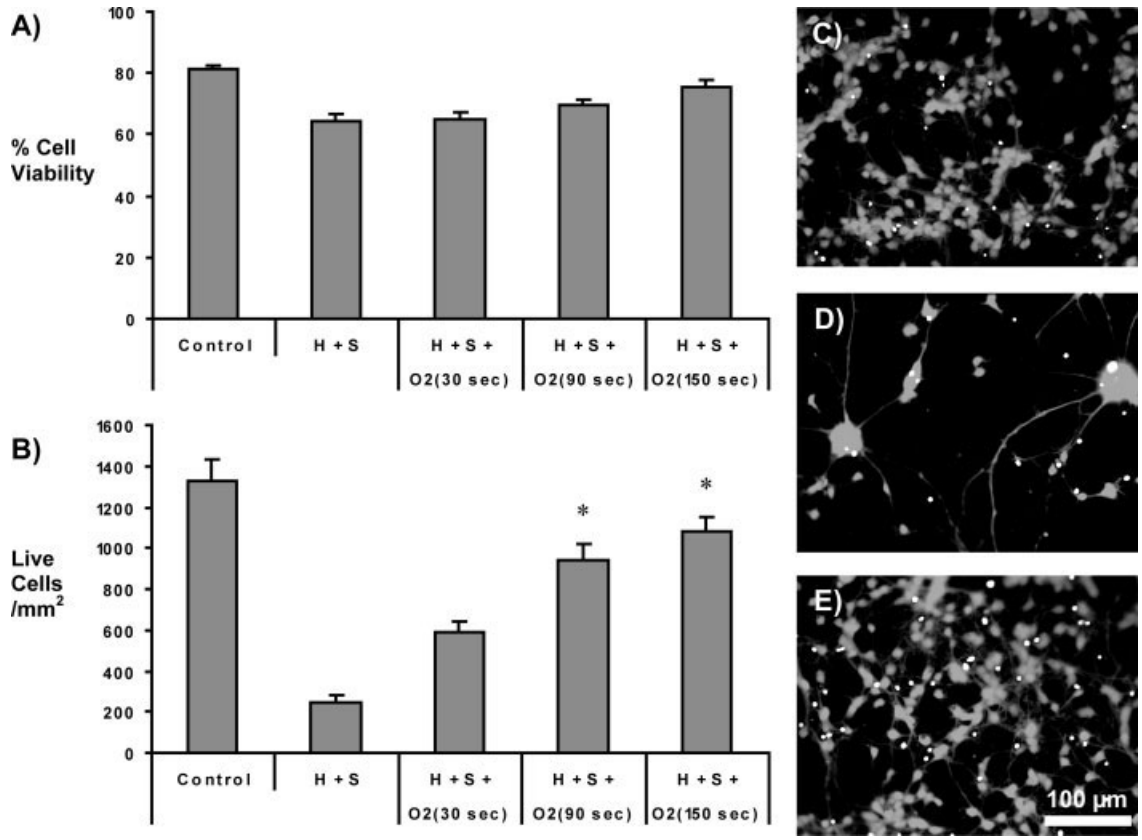

Figure 6. Assessment of different periods of glow discharge (oxygen plasma) treatment for improving cytocompatibility of SU-8 2000 samples. Neurons plated on top of heat plus isopropanol sonicated SU-8 2000 samples with or without glow discharge treatment were quantified as % cell viability (A) and live cells/mm^2 (B). Representative photomicrographs of neural cultures are shown on the right for cells plated on glass controls (Control, C), on heat plus isopropanol sonicated SU-8 2000 samples (H + S, D), on H + S treated SU-8 2000 samples with 150 s of glow discharge (H + S + O_2 (150 s), E) labeling live cells (gray) and dead cell nuclei (white). Live cell densities for the glow discharge treatments at 90 s (H + S + O_2 (90 s)) and 150 s (H + S + O_2 (150 s)) were significantly better than the other treatments but were statistically indistinguishable from each other (*$p < 0.05$).

ence was reduced when the baking temperature reached 150°C.[43]

The improved cytocompatibility of SU-8 2000 after isopropanol ultrasonication treatment following the heat treatment may be partially due to the high solvating capability of isopropanol, along with the increased kinetic energy for diffusion provided by ultrasonication, further helping in the removal of toxic leachants. The cured SU-8 2000 photoresist is highly crosslinked, resulting in a very low diffusion rate at the exposed surface under static conditions. Ultrasonication, not only may be increasing these diffusion rates, but also increasing turnover of the isopropanol solvating the leachants at the SU-8 2000 interface.[5]

Parylene coating helps improve the live cell density on the SU-8 2000, presumably by providing a barrier to the diffusion of leachants. This is supported by the XPS data for parylene coated SU-8 2000 where fluorine and antimony peaks are absent. While parylene by itself can not be used to make the high-aspect ratio MEMS structures, parylene coating may be useful especially for applications such as insulation on high-aspect ratio MEMS structures made out of SU-8 2000, because of ease in fabrication and its coating on SU-8 2000 enhances cell viability as compared to SU-8 2000 that is exposed to heat and sonication alone. Glow discharge treatment (oxygen plasma) was very effective in making SU-8 2000 more cytocompatible, consistent with the reported effects of wet chemical treatments.[19,44] Such treatments essentially make the surface more hydrophilic by increasing oxygen species (as indicated both by the water contact angle and XPS measurements), allowing better cell adhesion. The oxygen plasma treatment is not only effective at creating oxygen rich groups, but also etches the surfaces, thereby removing possible toxic leachants as well as roughing the surface,[1] which may improve cell attachment. While oxygen plasma treatment appeared to expose new fluorine and antimony, the fluorine levels were much lower than those associated with sonication alone (2% as compared to 20%). Despite, these low levels of fluorine and the

TABLE III
Assessment of the Surface Elemental Composition of SU-8 2000 Samples with the Various Treatments Tested in This Study Using X-ray Photoelectron Spectroscopy

Treatments	Elemental Composition						O %/C % Ratio
	C %	O %	F %	Si %	Sb %	Cl %	
Untreated	82	16	2	0	0	0	0.2
H	78	17	1	5	0	0	0.2
S	66	14	20	0	0	0	0.2
H + S	83	17	0	0	0	0	0.2
H + S + O_2	37	54	2	0	8	0	1.4
H + S + P	86	0	0	0	0	14	0.0
H + S + P + O_2	67	29	0	0	0	5	0.4

Most treatments did not affect the O/C ratio, however, the glow discharge (oxygen plasma) treatment significantly increased this ratio. Presence of fluorine as well as antimony was revealed in the SU-8 2000.

exposed antimony detected after the oxygen plasma treatment (which, probably get washed away during the poly-D-lysine coating prior to cell culture), the concomitant good neuronal survival and outgrowth suggest that it is the large increase in oxygen (0.2–1.4 oxygen to carbon ratio), which may increase surface hydroxyl groups, contributing to better poly-lysine binding to the surface and improved cellular adhesion,[45,46] that has a strong influence in determining neuronal viability. In addition, since XPS probes up to a few nanometers below the surface contacted by the cells, the depth of the XPS probe is likely to be greater than the level sensed by cells.

We also observed that UV exposure exacerbated SU-8 2000 cytotoxicity, instead of reducing it, as expected. This suggests that the UV exposure possibly increases at least some of the toxic leachants. The excess of strong acids generated by the photo acid generator after UV exposure[43] may contribute to the enhanced cytotoxicity after UV flood exposure. The contribution of the UV used in sterilization to this toxicity is expected to be minimal because the UV dose from the biohazard hood UV lamp (used for sterilization, $\leq$0.6 J/cm^2) is much lower than that of the mask aligner (used for UV treatment, 90 J/cm^2) and because the distance of the samples from the UV lamp while sterilizing ($\geq$65 cm) is much greater than that of the UV source in the mask aligner (7 cm). The detection on SU-8 2000 by XPS of fluorine and antimony, both photoacid generator associated elements, supports this conclusion. Likewise, CO_2 supercritical extraction also exacerbated toxicity of the SU-8 2000 rather than improving it. CO_2 treatment altered the physical characteristics of the SU-8 2000, resulting in sample warping.

The thick high-aspect ratio SU-8 2000 structures that we fabricated required longer prebake times to remove excess solvent for obtaining a similar solvent as a thinner film, higher exposure energies to ensure that photoacid generation with the UV was complete for the thick SU-8 structures, and longer postbake times to allow for completion of the photocrosslinking reaction, than recommended by the manufacturer.[20–25] To get a vertical wall profile, especially in high-aspect ratio structures, the amount of UV exposure and heat energy were increased. Based on Fick's law of diffusion, the thicker the SU-8 2000 material the tougher it would to detoxify. Therefore, we have included the 3 day heat and the 30 min sonication times which are long periods of times for diffusive removal. However, as discussed earlier, in practice as the thickness of the photoresist increases, its behavior with respect to processing conditions is different; different thickness of SU-8 2000 are obtained from different formulations of SU-8 2000, leading to fabrication procedures that are different for the different thicknesses in terms of the pre and post bake times, and exposure energies which do not necessarily scale linearly.[20,22,47] These differences can lead to the end materials with different amounts of possible toxic leaching materials, based on the crosslinking reaction progress and the crosslinking density. There are several varying processing parameters reported in the literature such as soft bake, exposure and post bake, which will contribute differently to the properties of the SU-8[47]/SU-8 2000.[22] The cytocompatibility of SU-8/SU-8 2000 is directly related to the fabrication process and the processing environment and these protocol changes will require ongoing cytocompatibility evaluation. The treatments examined here offer several options for thick, high-aspect ratio SU-8 2000 applications.

CONCLUSION

This study is the first characterization of SU-8 2000 cytotoxicity to neuronal cultures, linking the poor SU-8 2000 cytotoxicity to components within the SU-8 2000, such as solvents and photo acid generator associated elements and the poor adhesive

properties of SU-8 2000. Furthermore, this work demonstrates that postprocessing strategies for making SU-8 2000 more cytocompatible should include a combination of heat and isopropanol sonication followed by surface treatments of either oxygen plasma or parylene coating. In combination, these results provide a methodology for increasing the potential for SU-8 2000 to be a primary microfabrication material for bioMEMS and other biomedical applications.

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References

1. Voldman J, Gray ML, Schmidt MA. Microfabrication in biology and medicine. Annu Rev Biomed Eng 1999;1:401–425.
2. Gelorme JD, Cox RJ, Gutierrez SAR. Photoresist composition and printed circuit boards and packages made therewith. U.S. Pat. 4,882,245 (1989).
3. Shaw JM, Gelorme JD, LaBianca NC, Conley WE, Holmes SJ. Negative photoresists for optical lithography. IBM J Res Dev 1997;41:81–94.
4. Shaw M, Nawrocki D, Hurditch R, Johnson D. Improving the process capability of SU8. Microsyst Technol 2003;10:1–6.
5. Lorenz H, Despont M, Fahrni N, Brugger J, Vettiger P, Renaud P. High-aspect-ratio, ultrathick, negative-tone near-UV photoresist and its applications for MEMS. Sens Actuators A 1998;64:33–39.
6. Kotzar G, Freas M, Abel P, Fleischman A, Roy S, Zorman C, Moran JM, Melzak J. Evaluation of MEMS materials of construction for implantable medical devices. Biomaterials 2002;23: 2737–2750.
7. Song Y, Kumar CSSR, Hormes J. Fabrication of an SU-8 based microfluidic reactor on a PEEK substrate sealed by a 'flexible semi-solid transfer' (FST) process. J Micromech Microeng 2004; 14:932–940.
8. Heuschkel MO, Guerin L, Buisson B, Bertrand D, Renaud P. Buried microchannels in photopolymer for delivering of solutions to neurons in a network. Sens Actuators B 1998;48:356–361.
9. Voskerician G, Shive MS, Shawgo RS, Recum HV, Anderson JM, Cima MJ, Langer R. Biocompatibility and biofouling of MEMS drug delivery devices. Biomaterials 2003;24:1959–1967.
10. Golmieh G, Soussou W, Courellis S, Marmarelis V, Berger T, Baudry M. A biosensor for detecting changes in cognitive processing based on nonlinear systems analysis. Biosens Bioelectron 2001;16:491–501.
11. Golmieh G, Soussou W, Han M, Ahuja A, Hsiao M-C, Song D, Tanguay AR, Berger TW. Custom-designed high-density conformal planar multielectrode arrays for brain slice electrophysiology. J Neurosci Methods 2006;152:116–129.
12. Heuschkel MO, Fejtl M, Raggenbass M, Bertrand D, Renaud P. A three-dimensional multi-electrode array for multi-site stimulation and recording in acute brain slices. J Neurosci Methods 2002;114:135–148.
13. Tscherter A, Heuschkel MO, Renaud P, Streit J. Spatiotemporal characterization of rhythmic activity in rat spinal cord slice cultures. Eur J Neurosci 2001;14:179–190.
14. Streit J, Tscherter A, Heuschkel MO, Renaud P. The generation of rhythmic activity in dissociated cultures of rat spinal cord 2001;14:191–202.
15. Wirth C, Lüscher H-R. Spatiotemporal evolution of excitation and inhibition in the rat barrel cortex investigated with multielectrode arrays. J Neurophysiol 2004;91:1635–1647.
16. Berdondini L, Chiappalone M, van der Wal PD, Imfeld K, de Rooij NF, Koudelka-Hep M, Tedesco M, Martinoia S, van Pelt J, le Masson G, Garenne A. A microelectrode array (MEA) integrated with clustering structures for investigating *in vitro* neurodynamics in confined interconnected sub-populations of neurons. Sens Actuators B 2006;114:530–541.
17. Wu Z-Z, Zhao Y, Kisaalita WS. Interfacing SH-SY5Y human neuroblastoma cells with SU-8 2000 microstructures. Colloids Surf B: Biointerfaces 2006;52:14–21.
18. Weisenberg BA, Mooradian DL. Hemocompatibility of materials used in microelectromechanical systems: Platelet adhesion and morphology *in vitro*. J Biomed Mater Res 2002; 60:283–291.
19. Wang Z, Stangegaard M, Dufva M, Kutter JP, Wolff A. A simple hydrophilic treatment of SU-8 surfaces for cell culturing and cell patterning. In Ninth International Conference on Miniaturized Systems for Chemistry and Life Sciences, October 9–13, 2005, Boston, Massachusetts, p 745–747.
20. Nano™ SU-8 2000 Negative Tone Photoresist Formulations 2035–2100, Microchem, 1254 Chestnut Street, Newton, MA 02464. Available at: www.microchem.com/products/su_eight.htm.
21. Choi YC, McClain MA, LaPlaca MC, Frazier AB, Allen MG. Three dimensional MEMS microfluidic perfusion system for thick brain slice cultures. Biomed Microdevices 2007;9:7–13.
22. Barber RL, Ghantasala MK, Divan R, Vora KD, Harvey EC, Mancini DC. Optimisation of SU-8 2000 processing parameters for deep X-ray lithography. Microsystem Technologies 2005;11:303–310.
23. Ho C-H, Chin K-P, Yang C-R, Wu H-M, Chen S-L. Ultrathick SU-8 2000 mold formation and removal, and its application to the fabrication of LIGA-like micromotors with embedded roots. Sens Actuators A 2002;102:130–138.
24. Lin C-H, Lee G-B, Chang B-W, Chang G-L. A new fabrication process for ultra-thick microfluidic microstructures utilizing SU-8 2000 photoresist. J Micromech Microeng 2002;12:590–597.
25. Bogdanov AL, Peredkov SS. Use of SU-8 2000 photoresist for very high-aspect ratio x-ray lithography. Microelectron Eng 2000;53:493–496.
26. Wagenaar DA, Pine J, Potter SM. An extremely rich repertoire of bursting patterns during the development of cortical cultures. BMC Neurosci 2006;7:1–18.
27. Voigt T, Baier H, de Lima AD. Synchronization of neuronal activity promotes survival of individual rat neocortical neurons in early development. Eur J Neurosci 1997;9:990–999.
28. Gross GW, Kowalski JM. Origins of activity patterns in self-organizing neuronal networks *in vitro*. J Intell Mater Syst Struct 1999;10:558–564.
29. Brewer GJ, Torricelli JR, Evege EK, Price PJ. Optimized survival of hippocampal neurons in B27-supplemented neurobasal™, a new serum-free medium combination. J Neurosci Res 1993;35:567–576.
30. Brewer GJ. Isolation and culture of adult rat hippocampal neurons. J Neurosci Methods 1997;71:143–155.
31. Brown SA. Introduction. In: Brown SA, editor. Cell-Culture Test Methods. ASTM Special Technical Publication 810. Philadelphia: ASTM; 1983. p 2–3.
32. Lontz JF, Nadijcka MD, Holmes R. Assessment of biocompatibility of orofacial materials and devices by culturing with

human excised donor tissues. In: Brown SA, editor. Cell-Culture Test Methods. ASTM Special Technical Publication 810. Philadelphia: ASTM; 1983. p 77–87.
33. Lesuisse C, Martin LJ. Long-term culture of mouse cortical neurons as a model for neuronal development, aging, and death. J Neurobiol 2002;51:9–23.
34. Beche E, Berjoan R, Viard J, Cros B, Durand J. XPS and AES characterization of SiN_x:H layers deposited by PECVD on Parylene C. Effects of thermal treatments on Parylene C surfaces and Parylene C/SiN_x:H interlayers. Thin Solid Films 1995;258:143–150.
35. Johnson DW, Nawrocki DJ, Ruhmann R. Improving the process capability of SU-8, Part III. J Photopolym Sci Technol 2002;15:749–756.
36. Dentinger P, Krafick KL, Simison KL. Photoimageable composition. U.S. Pat. 6,858,378 B1 (2005).
37. Aktary M, Jensen MO, Westra KL, Brett MJ, Freeman MR. High-resolution pattern generation using the epoxy novolak SU-8 2000 resist by electronbeam lithography. J Vac Sci Technol B 2003;21:L5–L7.
38. Yang H, Quan H, Tamura M, Sekiya A. Investigation into antimony pentafluoride-based catalyst in preparing organo-fluorine compounds. J Mol Catal A 2005;233:99–104.
39. Horbet TA, Ratner BD, Schakenraad JM, Schoen FJ. Biology, biochemistry, and medicine: Some background concepts. In Ratner BD, Hoffman AS, Schoen FJ, Lemons JE, editors. Biomaterial Science: An Introduction to Materials in Medicine. San Diego: Academic Press; 1996. p 133–141.
40. Zhang J, Zhou WX, Chan-Park MB, Conner SR. Argon plasma modification of SU-8 for very high-aspect ratio and dense copper electroforming. J Electrochem Soc 2005;152: C716–C721.
41. Musiek ES, Breeding RS, Milne GL, Zanoni G, Morrow JD, Mclaughlin BA. Cyclopentenone isoprostanes are novel bioactive products of lipid oxidation which enhance neurodegeneration. J Neurochem 2006;97:1301–1313.
42. Kirman CR, Sweeney LM, Corley R, Gargas ML. Using physiologically-based pharmacokinetic modeling to address nonlinear kinetics and changes in rodent physiology and metabolism due to aging and adaptation in deriving reference values for propylene glycol methyl ether and propylene glycol methyl ether acetate. Risk Anal 2005;25:271–284.
43. Feng R, Farris RJ. Influence of processing conditions on the thermal and mechanical properties of SU8 negative photoresist coatings. J Micromech Microeng 2003;13:80–88.
44. Nordstrom M, Marie R, Calleja M, Boisen A. Rendering SU-8 hydrophilic to facilitate use in micro channel fabrication. J Micromech Microeng 2004;14:1614–1617.
45. Curtis AS, Forrester JV, McInnes C, Lawrie F. Adhesion of cells to polystyrene surfaces. J Cell Biol 1983;97:1500–1506.
46. Keselowsky BG, Collard DM, Garcia AJ. Surface chemistry modulates focal adhesion composition and signaling through changes in integrin binding. Biomaterials 2004;25:5947–5954.
47. Zhang J, Tan KL, Hong GD, Yang LJ, Gong HQ. Polymerization optimization of SU-8 photoresist and its applications in microfluidic systems and MEMS. J Micromech Microeng 2001;11:20–26.