Assessment of the biocompatibility of photosensitive polyimide for implantable medical device use

Y. Sun,[1] S. P. Lacour,[1] R. A. Brooks,[2] N. Rushton,[2] J. Fawcett,[3] R. E. Cameron[1]
[1]*Department of Materials Science and Metallurgy, University of Cambridge, Pembroke Street, Cambridge CB2 3QZ, United Kingdom*
[2]*Orthopaedic Research Unit, University of Cambridge, Box 180, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, United Kingdom*
[3]*Cambridge Centre for Brain Repair, University of Cambridge, Forvie Site, Robinson Way, CB2 2PY, United Kingdom*

Received 21 December 2007; revised 31 March 2008; accepted 2 April 2008
Published online 18 June 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.32125

Abstract: Polyimides have been widely used for biosensor encapsulation and more recently as substrates for neural implants. They have excellent thermal stability, high chemical resistance, and can be prepared as thin, flexible films. Photosensitive polyimides present similar physical properties to polyimides, and have the advantage that they can be photo-lithographically patterned. However, to date little data on their biocompatibility has been reported. Two commercially available polyimides (PI) and one photo-sensitive polyimide (PSPI) were evaluated *in vitro* using the ISO 10993 standard on biocompatibility. The materials were Dupont Kapton foil HN, HD Microsystem PI2611, and Fujifilm Durimide 7020 (PSPI). PI2611 and Durimide 7020 were spin-coated on silicon wafers, cured at temperatures ranging from 150 to 450°C, and sterilized by autoclave. All materials were evaluated using a scanning electron microscope pre- and postcell culture. Cell viability was determined by an MTS assay. Their mechanical properties and stability during cell culture as a function of time and environment were investigated by nanoindentation. The MTS results show that PSPI is noncytotoxic compared with the negative control of polyethylene and the conventional PIs tested. Fibroblast adhesion, morphology, and spreading were good and better on the PSPI substrate than on the PI2611. Schwann cell appearance was similar on each of the PIs and the PSPI tested. The results suggest that PSPIs may have potential use for biological microsystem and neuroprosthetic applications. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res 90A: 648–655, 2009

Key words: photosensitive polyimide; biocompatibility; cell culture; fibroblast; Schwann cells

INTRODUCTION

Polymers are widely used for medical devices and implants. They can be employed as substrate and/or encapsulation materials, be processed into complex shaped devices, and may even be biochemically functionalized. Biomedical polymers must be biocompatible, that is, they should not cause adverse biological effects to the surrounding tissues nor degrade during long-term exposure to the ionic biological environment. In this article, we report on the *in vitro* biostability and cytotoxicity of photosensitive polyimide (PSPI). Data were collected following the ISO10993 "Biological evaluation of medical devices" protocol.[1]

Correspondence to: Y. Sun; e-mail: ys302@cam.ac.uk
Contract grant sponsor: EPSRC-MRC

Polyimides are high-performance polymers. They are strong (Young's modulus ~3 GPa), chemically inert and resistant, electrically insulating, and are fully compatible with device fabrication processes.[2–5] There are two types of polyimides: traditional non-photosensitive polyamide (PI) based on poly (amic acid) precursors, and photosensitive polyamide (PSPI) based on poly (amic acid ester)s or salt precursors.[6] PI and PSPI are typically used in liquid form, spin-coated on the substrate, and thermally cured into a film. PI films may be patterned using photolithography and dry/wet etching. PSPI films are directly patterned by UV insulation and development, which significantly reduces the number of process steps, and enables the fabrication of multilayered, 3D structures.

PIs support cell growth Lee et al. reported that fibroblast cells attached, spread and grew on PI electrode surfaces similarly to those cultured on tissue culture polystyrene controls.[7] PI is often used to coat silicon-based implants[8–10] providing both a chemical

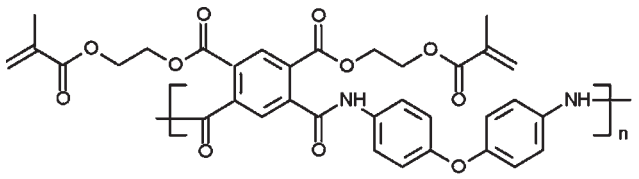

Figure 1. Structure of photosensitive polyimide precursor (data supplied by Fujifilm electronic materials).

barrier and an electrical insulation layer.[6,11,12] Flexible PI-based microdevices have been interfaced with nerves as neural prostheses, and show excellent properties as implant material[13,14] with chronic stability as they were intact after several months of implantation.[15–17] The flexibility of polyimide may decrease the mechanical impedance mismatch between the rigid electrode and the soft tissue therefore reducing tissue damage because of electrode micromotion.[18–20]

The use of PSPI for biomedical implants is a recent development Rousche et al. have successfully designed and fabricated PSPI/PI based cortical electrodes.[19] Tan and Saltzman reported on *in vitro* neurite growth through arrays of PSPI ridges on glass.[11,14] But to our knowledge, no report addressing PSPI biocompatibility has been published.

The PSPI chosen in this study is Durimide® 7020 from Fujiifilm, the structure of which is given in Figure 1. During thermal imidization, the photoactive groups debond from the polymer backbone by imide-ring closure and are degassed from the resulting PSPI films. These groups may change the imidization kinetics of the precursors as well as the morphological structure in the condensed state, leading to different structures and properties from the corresponding PI film.[13] The bioresponse may therefore not be the same and a full assessment of the biocompatibility of photosensitive PI is required.

We have investigated the *in vitro* biocompatibility of PSPI films in comparison with spinnable PI and extruded PI (Kapton®) films. We have evaluated their biostability when exposed to ionic buffer solution, characterized the films using nanoindentation, and studied their cytotoxic effects to mouse fibroblast L292 and rat primary Schawnn cells. Our results show that PI and PSPI materials are not altered by long-term exposure to saline solution, and do not induce adverse biological effects in these tests.

EXPERIMENTAL PROCEDURES

Materials and film preparation

Two PI materials were investigated: Dupont Kapton® HN (extruded film), and Dupont spinnable PI 2611. Photosensitive polyimide was purchased from FujiFilm (Durimide 7020).

All precursor solutions were stored in a freezer and used as supplied. The solutions were left at room temperature for 4 h to thaw prior to use.

The films were spin-coated onto silicon wafers preliminary cleaned by sonication in acetone, rinsed in isopropanol and exposed to UV ozone for 5 min. The films were then soft baked at 65°C for 5 min followed by 110°C for 5 min and then 65°C for 5 min on a hot plate. The soft baked samples were polymerized by curing for 1 h in a nitrogen atmosphere. Curing temperatures ranging from 150 to 450°C were tested. Finally the PI and PSPI films were peeled off the Si wafer. The thickness of cured films was ~20 μm.

Thermogravimetry analysis

Solvent residues in the PI and PSPI films were evaluated by TGA (TA Instruments Q500 TGA, US) with a ramping rate of 10°C/min under nitrogen from room temperature to 600°C. Sample weight-loss in percent was recorded as a function of temperature.

Equilibrium water content

Swelling of the polyimide films was determined gravimetrically by measuring the weight gain of the sample as a function of time in water at 37°C. Measurements were taken until the equilibrium was reached, that is when three consecutive determinations gave the same weight. The time to reach swelling equilibrium was 1 week for all the samples. The equilibrium water content (EWC) was calculated as the differential mass ratio of water to hydrated sample according to Eq. (1). EWC was calculated as the mean of at least six measurements of different samples.

$$EWC = \frac{(W_{wet} - W_{dry})}{W_{wet}} \times 100\% \quad (1)$$

Cell culture

L929 Mouse fibroblast cells were cultured in Dulbeccos' Modified Eagles Medium (DMEM) (Sigma, UK) supplemented with 10% (v/v) foetal calf serum (FCS) and 1% (v/v) L-glutamine at 37ºC in an incubator gassed with 5% CO_2 for 1–4 days depending on cell confluency. Cells were harvested for passage or experiments using a solution containing 0.5 mg mL^{-1} trypsin and 0.2 mg mL^{-1} ethylenediaminetetraacetic acid (EDTA) (Sigma, UK), and were used between passages 4–10 at ~80% confluence.

The fibroblasts were detached from a 75 cm^2 T-flask by using trypsin-EDTA and the detached cells were resuspended in a few millilitres (the volume varied with the total number of cells) of cell culture medium. Cell counting was performed and the cell suspension was diluted with medium if necessary. The cell suspension was added to a 24-well cell culture plate (Corning, UK) and 1 mL of cell culture medium was subsequently pipetted into the wells

to give cell concentrations of 5 × 104 cells/well. Cultures were incubated at 37°C gassed with 5% CO_2 for 3 days.

Sample extraction

In this part of study the short-term effect of any material leaching from the substrate was simulated by exposing fibroblast cells to the extract of the materials according to ISO 10993.

Three pieces of each specimen (12 × 12 mm^2) were autoclaved at 120°C for 20 min. Extracts were obtained by placing the samples in cell culture medium (DMEM with 10% serum as specified by the ISO 10993 standard) for 24 h at 37°C. The resulting fluid extract was then poured on a cultured-cell monolayer, replacing the medium that had nourished the cells to that point. By doing so, cells were supplied with a fresh nutrient medium containing extractables derived from the specimens. The cultures were then incubated at 37°C gassed with 5% CO_2 for 2 days.

MTS assay

To assess cell viability, the CellTiter 96® nonradioactive MTS-based cell proliferation assay was applied (CellTiter 96® Aqueous One Solution Reagent, Promega, USA). The colorimetric MTS assay is based on the selective ability of living cells to reduce 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium bromide into a colored formazan product that is soluble in cell culture medium. The quantity of produced formazan was measured by recording the absorbance at 492 nm, which is directly proportional to the number of living cells.

Briefly, after cell culture, the medium was removed and the cells were washed thrice with phosphate buffered saline (PBS). Immediately after removal of PBS, 200 μL of MTS solution was pipetted into each well containing 1 ml of DMEM. After 1 h incubation at 37°C gassed with 5% CO_2,the supernatant of all wells were respectively aspirated and measured on an multiwell plate reader (BMG LABTECH FLUO star OPTIMA) at 492 nm (OD) with a subtraction of the absorbance of cell-free blank volume at 492 nm. Cell number was determined using a linear correlation between absorbance and cell concentration. The results from three individual experiments were averaged.

Scanning electron microscopy

Cell suspensions (~5 × 104 cells/well) were seeded on the surface of the polyimide films. After one-day incubation allowing for cell attachment and spreading, the samples were rinsed to remove extraneous debris, unattached cells, and/or proteins, and primarily fixed in 4% glutaraldehyde in 0.1*M* PIPES buffer. Then the samples were washed in 0.1*M* HEPES buffer and incubated overnight to remove unreacted primary fixative. The samples were then placed in a secondary, fixative 1% osmium tetroxide (OsO_4), for 2 h, dehydrated in a graduated ethanol series, and critical point dried (Polaron E3000 CPD, Bal-Tec, France). The fibroblasts on polyimide films were examined on a Philips XL30 FEG scanning electron microscope using an accelerating voltage of 5 kV, after sputtering of a thin gold layer (Polaron E5000 Sputter Coater). Micrographs were taken randomly on the different surfaces and for each sample the test was repeated at least three times with cells from different spots. Thus, the results represent a general population of cells from the same sample.

Mechanical testing

The mechanical properties of PI and PSPI films were measured using an ultra-low load indentation system, NANOINDENTER® XP (MTS, US), equipped with a Berkovich diamond indenter tip and tip geometry calibration was made by using standard fused silica. Continuous stiffness measurement (CSM) technique was applied to record the sample's Young's modulus as a function of indentation depth. The indentation locations on each specimen were manually selected, and a series of 10 indentations per specimen were made to obtain a mean Young's modulus. All specimens were 20 μm think with a length of 10 mm and a width of 5 mm.

To investigate the effect of water on the mechanical properties of the polyimides, the specimens were incubated in 100 mL PBS at 37°C. The solution was renewed every 7 days for 6 months. Nanoindentation was carried out while the specimens were immersed in PBS to study *in situ* effects, as shown in Figure 2.[21]

Statistical analysis

All results are expressed as mean ± SD. Paired *t* test was used to evaluate the statistical significance of measured differences between materials. A 95% confidence level was selected to define significance for all statistical tests, and differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Mechanical testing analysis

All tested polyimides had low water absorption (<2 wt %) and swelling of the PIs was not obvious visually. The TGA curve as demonstrated in Figure 3 shows the thermal stability of the fully cured PSPI film. Loss occurred (2 wt%) during heating progress up to 500°C. Thermal degradation took place above 600°C. This result confirms the high thermal resistance of PSPI.

The change in Young's modulus as a function of incubation time in PBS is given in Figure 4. All the polyimides had a Young's modulus of the order of 3–6 GPa. We observed a drop in modulus for all samples immersed in saline solution within the first 2 weeks, which then leveled over the remaining

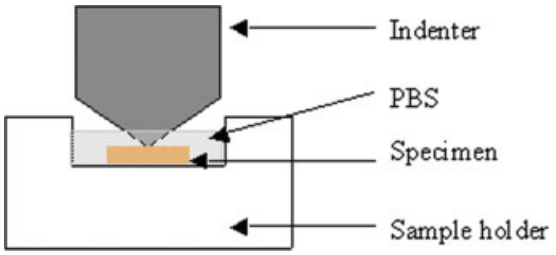

Figure 2. Schematic illustration of nanoindentation test. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

incubation period (24 weeks). PSPI 7020 showed the smallest percentage of drop in mechanical Young's modulus ($\Delta E/E$) in PBS (5.4%), followed by PI2611 (7.3%), while KHN exhibited the largest percentage decrease (7.7%). PSPI7020 therefore showed the best overall resistance to water with little change in mechanical properties. KHN showed the greatest effect, suggesting that water permeability may be an important factor when considering KHN mechanical properties.

To summarize, PSPI7020 has been shown to be mechanically stable after long term *in vitro* testing.

Cytotoxicity testing

In this study, the ISO standard biological evaluation of medical devices test protocol for *in vitro* cytotoxicity, ISO-10993-5 was applied.[1] The cytotoxicity of PI and PSPI extraction products when added to L929 mouse fibroblast cells *in vitro* was studied using the MTS assay.

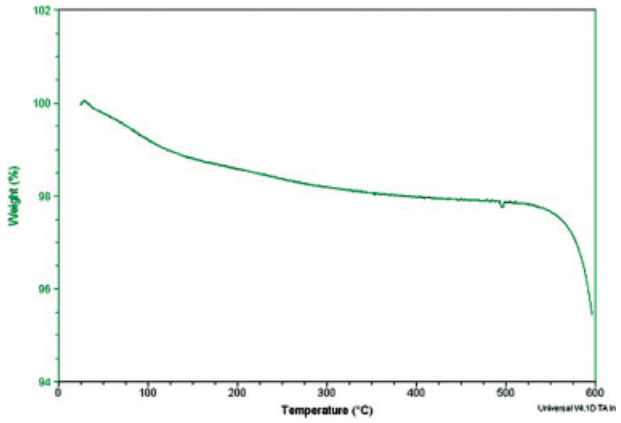

Figure 3. TGA curve of a 20 μm thick PSPI film with a heating rate of 10°C/min under nitrogen. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

All PI and PSPI samples were fully cured at 400°C for 1 h. Figure 5 shows L929 fibroblast cell numbers following culture with each sample extract. Cells were also cultured in a polyethylene extraction as a negative control.

We observed that the L929 fibroblast cell viability in all sample extractions were not significantly dif-

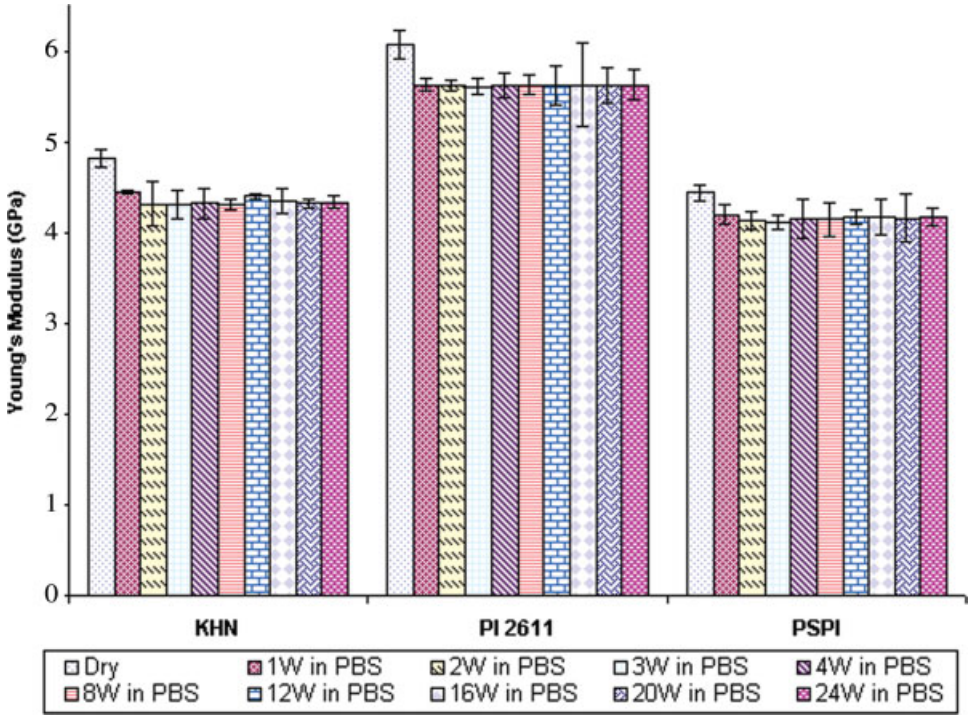

Figure 4. Nanoindentation results of specimen in different incubation period. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

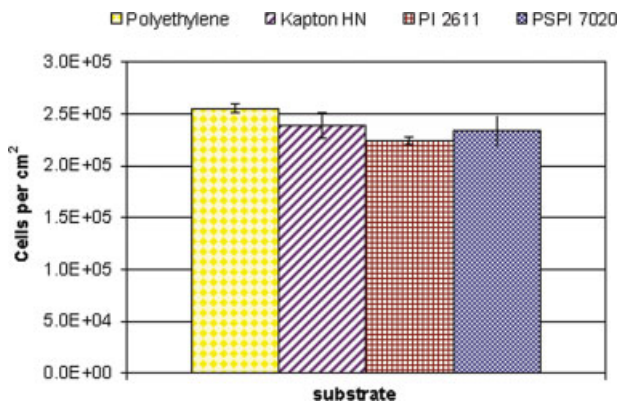

Figure 5. MTS results of cytotoxicity testing on polyethylene (negative control), Kapton HN, PI 2611 and PSPI 7020. Results were expressed in cells/cm^2. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ferent from those of cells cultured in polyethylene extraction. Therefore fully cured PI and PSPI films demonstrate no significant cytotoxicity on fibroblast cell growth.

Scanning electron microscopical observation

We used SEM imaging to monitor cell morphology, adhesion, and spreading on the various substrates. We examined two cell types, 24 h after seeding on the samples: mouse L929 fibroblasts from a cell line, and rat primary Schwann cells. Unattached cells in suspension are typically spherical in shape. In this rounded state, the surface to volume ratio of the cell is severely reduced, cell proliferation ceases, and the supply of nutrients is decreased. Therefore, nonspreading of cells is an undesirable property of a supposedly cell adhesive material. SEM was performed to examine the morphology of both fibroblast cells and Schwann cells attaching and spreading on the tested surfaces after 24 h (Figs. 6 and 7).

Figure 6 presents fibroblast cells on PI and PSPI films after 24 h incubation. The highest density of cells was observed on KaptonHN. Cells exhibited a normal fibroblastic morphology, and adhered well to the surface of KaptonHN. The cells have a characteristic elongated and polygonal morphology, indicat-

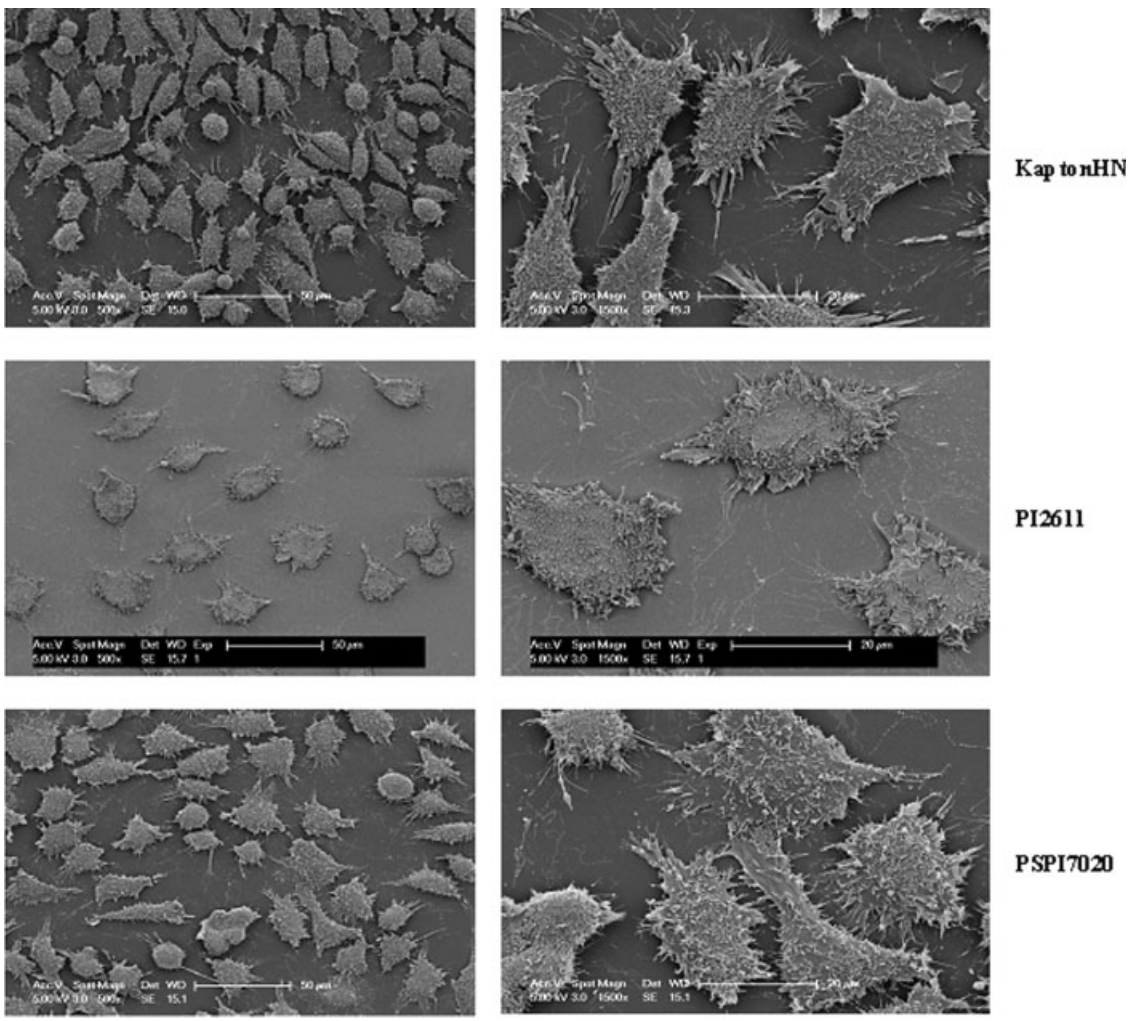

Figure 6. Scanning electron micrographs of L929 fibroblast cells cultured for 24 h on KaptonHN, PI 2611 and PSPI7020.

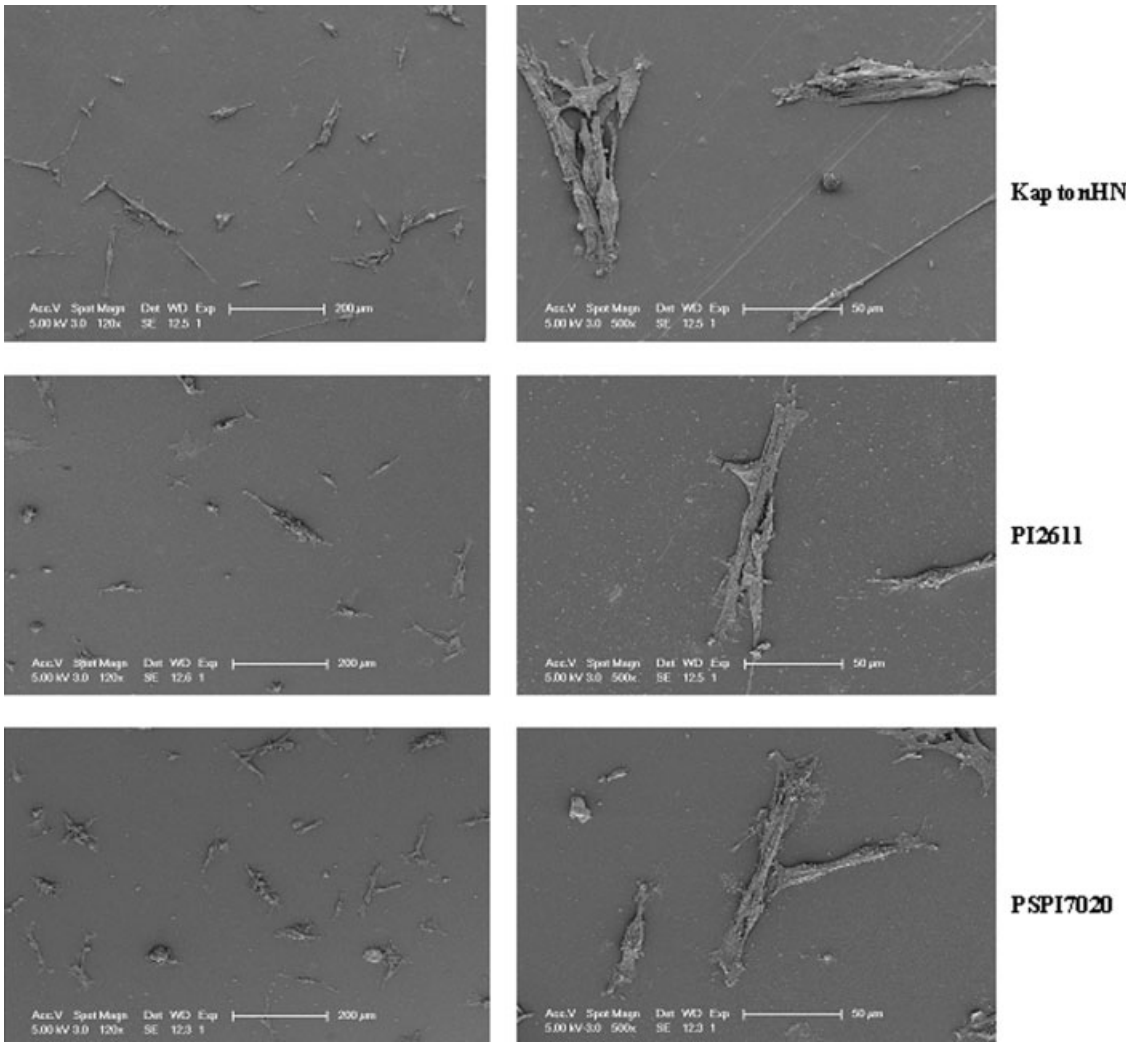

Figure 7. Scanning electron micrographs of Schwann cells cultured for 24 h on KaptonHN, PI2611, and PSPI7020.

ing that they are able to attach and have begun to spread out on the surface. On PSPI7020, the fibroblast cells adhered intimately to the surface, spread very well, grew in a flat monolayer with no clear orientation and exhibited extensive filamentous extensions (filopodia) and lamellipodia (extended flat regions of the cytoplasm), both of which are indicators of healthy cells. On PI2611, although individual cells were spreading on the sample surface, only a few cells adhered to the surface. The cells had a more rounded morphology with little signs of filopodia extension. This suggests poor viability of the fibroblasts on the PI film.

However, as demonstrated in Figure 7, all the tested candidate materials provided equally good surfaces for Schwann cell attachment and spreading.

From this study, we can conclude that surfaces of KaptonHN and PSPI7020 are equivalently good for cell spreading, however, surfaces of PI2611 were less favored by fibroblast cells. It was shown that the behavior of cells on materials is linked to the nature of the materials and their surface properties. All these parameters can also affect final protein adsorption and thus cell adhesion, and must be taken into account for choosing suitable substrate and encapsulation materials.

Another interesting phenomenon we noticed during this work was that, the curing temperature of tested samples has a remarkable effect on the morphologies of cell spreading and attachment. PI2611 was cured at 200 and 400°C respectively, and SEM observation shows the different cell behavior observed for fibroblast cells (Fig. 8).

Materials for biomedical implants have a bidirectional relationship with the biological tissues. While the material physical properties should not be altered by the biological environment, implant materials should not release adverse residues. The chemical stability of polyimide is only ensured when it is fully cured. At low curing temperature (e.g., 200°C),

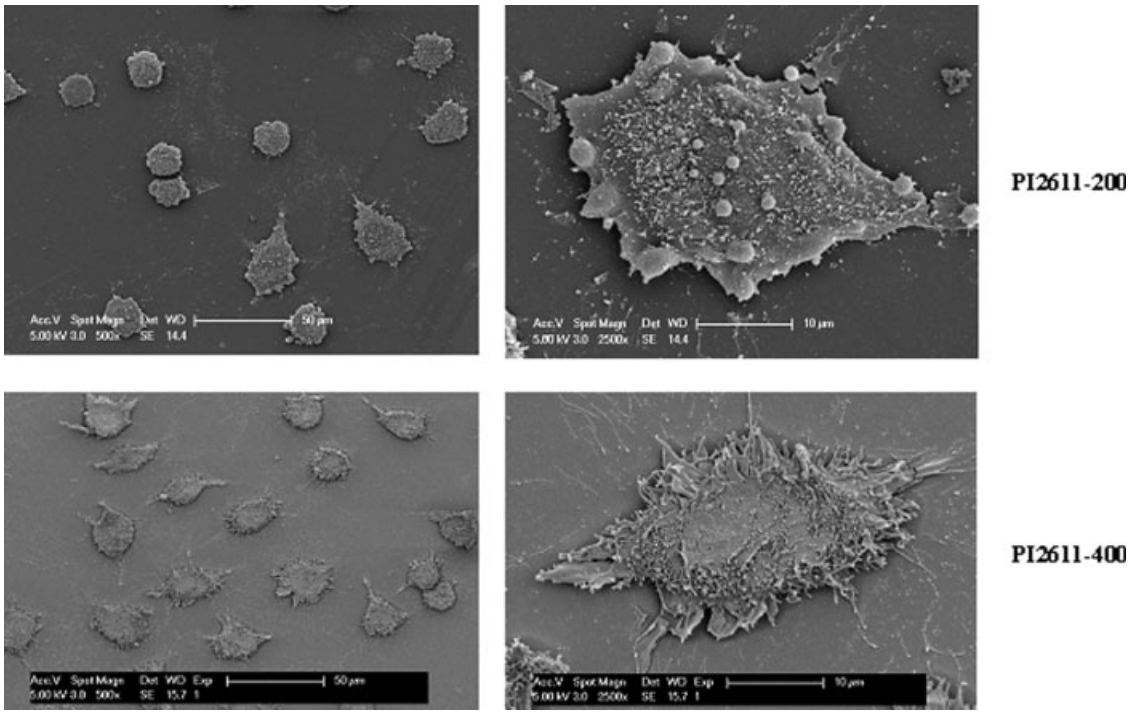

Figure 8. Scanning electron micrographs of fibroblast cells cultured for 24 h on PI2611 cured at 200°C (PI2611-200) and 400°C (PI2611-400). Equation (1) calculation of equilibrium water content.

the imidization process is not complete; and chemical residues including polyamide acid may be released.

Figure 8 illustrates the effect of curing the potential implant material on fibroblast growth and proliferation. Samples of PI2611 were cured at 200 or 400°C. Fibroblasts cultured on such films show poor adhesion and viability, as shown Figure 8-top. The cells are round, isolated, and have very few projections on the surface. In contrast, the cells cultured on a fully cured PI (at 400°C) spread on the surface sending several filopodia. High adhesion of the cells on the material surface usually indicates good cell viability. It seems likely that chemical residues removed by the 400°C cure but not by the 200°C cure are responsible for the differences.

CONCLUSIONS

On the basis of the current study, we can conclude that the photosensitive polyimides studied here are suitable for application as a biomaterial since they are not cytotoxic and offer excellent long-term stability. Therefore, they are promising materials for the design of nerve conduits.

The authors thank Robert Cornell for technical assistance in TGA, Andrew Rayment for help in mechanical testing and Jeremy Skepper for assistance in the morphological studies.

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