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Biocompatibility of Te–As–Se glass fibers for cell-based bio-optic infrared sensors

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The chemical stability and toxicity of Te–As–Se (TAS) infrared fibers are investigated. These fibers are used for biosensing applications that involve direct contact with live cultivated human cells. It is shown that TAS fibers exhibit a small oxidation layer after extended exposure to air. This layer is highly soluble in water and easily removed. However, the TAS glass itself is stable in water over several days. While oxidized fibers release arsenate ions, which result in toxic effects to the cells, fresh or washed fibers show no toxic effects. A good correlation is shown between surface etching and the disappearance of toxicity.

I. INTRODUCTION

Chalocogenide glasses belong to a unique class of amorphous materials that can be molded or drawn into fibers while retaining wide transparency over the infrared (IR) region.[1,2] This combination of properties has led to a number of applications ranging from night vision optics,[1] to astronomy,[3] biomedical imaging,[4] environmental monitoring,[5] and fiber-based remote sensing of chemical and biological species.[6] In particular, fiberoptic sensing has been widely investigated due to the transparency of chalcogenide glasses in the vibrational domains of most organic species. The collection of vibrational spectra that is unique to each molecule allows highly specific recognition of chemical species. Measurements involving chalcogenide fibers are typically performed through short-term exposure (in minutes) to an analyte, such as biomedical analysis of diseased tissues,[7] or through medium-term exposure (a few days), as in pollutant monitoring in water wells.[8] This class of fibers is now becoming commercially available[9]; however, their toxicity and stability in conditions of normal usage remains nearly unknown.

When a beam of light propagates in an optical element, part of the electric field can extend outside of the material surface and interact with a compound that is in contact with the interface. This principle constitutes the basis of IR evanescent wave spectroscopy and, in particular, the widespread attenuated total reflectance spectroscopy. The same principle also applies to fibers, and permits remote IR spectroscopy to be performed with the appropriate IR-transparent fibers and a conventional Fourier transform IR spectrometer. The source light is coupled to one end of the fiber, and the resulting signal is collected at the other end. This technique is termed fiber evanescent wave spectroscopy (FEWS). This type of vibrational spectroscopy is nondestructive, highly selective, and relatively easy to perform. It has been used to detect the vibrational signatures of chemical and biological species, with high sensitivity in various environments.[6]

It has been shown that both the selectivity and sensitivity of this technique can be enhanced by functionalizing the surface of the optical element. This is achieved by either concentrating or selectively immobilizing specific analyte molecules at the fiber surface, where the evanescent wave is most intense.[10–14] More recently, IR fibers have been functionalized with living cells, which can act as selective recognition elements for the detection of a specific class of hazardous species.[15–17] It has been shown that the spectroscopic response of mammalian cells attached to a fiber surface can be monitored to sense the presence of toxic agents.[18] Furthermore, an analysis of the spectral variation can provide information on the mechanisms of cell damage. Cell-based sensors have received increasing attention recently due to their high sensitivity and selectivity in detecting biological hazards.[19–22] Sensors based on live human cells offer a means to directly quantify the health impact of environmental hazards. In this respect they provide an additional level of information beyond that achieved with

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conventional sensors, which merely detect the presence of a toxic species but report nothing on its activity.

Two types of IR fibers have been commonly used to perform FEWS measurements. Silver halide fibers have a wide transparency window but have a tendency to dissolve and deteriorate when in contact with biological materials.[23,24] Chalcogenide fibers, on the other hand, are considered to be chemically stable and have been used to monitor several kinds of biological processes.[7,25,26] Many chalcogenide glasses contain As to stabilize the covalent glass network. The presence of this highly toxic element can raise issues for biological sensing applications, because even a small amount of As can significantly alter the metabolic functions of living cells and tissues. Arsenic is a toxic metal known to cause a variety of effects on cells, including damaging DNA, leading to the induction of cytoprotective functions, and inhibiting cell proliferation and metabolic function.[27] It is therefore essential to ensure that the glass fiber is sufficiently stable and will not itself interfere with the measurement.

A detailed study of the chemical stability and toxicity of chalcogenide fibers during long-term storage in air and during short-term and medium-term exposure to aqueous environments has not been performed. Here, we present a study on the biocompatibility of As containing chalcogenide fibers and their chemical stability in various environments. Their effect on human cells is quantified and the consequences, for the design of cell-based IR sensors, are addressed.

II. EXPERIMENTAL PROCEDURES

A. Chalcogenide fibers

Glass preforms of composition $Te_2As_3Se_5$ [Te–As–Se (TAS)] were synthesized in high purity to obtain optical quality material, with low transmission losses, suitable for fiber processing. A detailed experimental description of this process has been reported elsewhere.[28,29] These fibers have an effective transmission window in the range of 2–12 μm and are drawn to a diameter of about 400 μm. The diameter is reduced to approximately 100 μm along the sensing zone to improve sensitivity in FEWS applications.[30]

The chemical stability of these fibers was evaluated by monitoring variations in the surface topology and chemical composition after exposure to air and aqueous solution. Atomic force microscopy (AFM) was used to characterize variations in surface topology and roughness. X-ray photoelectron spectroscopy (XPS) was used to characterize variations in chemical composition at the fiber surface. Inductively coupled plasma mass spectrometry (ICP-MS) was used to detect the presence of arsenic dissolved in the solution surrounding the fiber.

B. Cell characterization

The biocompatibility of TAS fibers was evaluated by characterizing both the extent of cell attachment to the fiber surface as well as the toxicity of the fibers toward live cells. The cells used in this study were A549 immortalized human lung cells that form a strong attachment to surfaces. Details concerning the culture of these cells have been reported elsewhere.[17]

The extent of cell coverage on the fiber surface was characterized by fluorescence imaging using the live/dead Reduced Biohazard Viability/Cytotoxicity Kit#1 from Molecular Probes, Inc., Eugene, OR. Viable cells were stained with the SYTO 10 green fluorescent nucleic acid stain, while nonviable cells were stained with the Dead Red nucleic acid dye. Optical bright-field images and fluorescence images of the same field of view were collected to evaluate cell coverage.

The toxicity of TAS fibers toward mammalian cells was investigated using the same A549 cell line as well as rat alveolar macrophage cells (NRL8686, obtained from American Tissue Type Culture Catalog). Cultures of both of these cell lines were exposed either directly to TAS fibers or to the solution in which the fibers had been soaked. Old, new, and washed fibers were tested. Fibers were soaked either in Dulbecco's Modified Eagle's cell culture media (Sigma Chemical Co., St. Louis, MO), phosphate-buffered saline (PBS) solution, or 0.9% NaCl aqueous solution for 1–24 h. To quantify the cell viability, the cell cultures were exposed to the various fibers and soaking solutions for 24 h, while incubating at 37 °C and 5% CO_2, before the cell response was quantified. A WST-1 (Takara Bio Inc., Otsu, Japan) assay that colorimetrically quantifies cellular activity was used to assess cell viability.[31]

III. RESULTS

A. Fiber toxicity toward live cells

During a typical live-cell biosensing experiment,[15] the sensing zone of the fiber is first introduced into a saline solution to collect background reference spectra. Then a centrifuged pellet of cultured cells is introduced into the solution and allowed for 1 h to attach as a monolayer to the fiber surface. Figure 1 shows a close-up view of a group of A549 cells forming a strong attachment to the surface of a TAS fiber. Strong contacts are important in FEWS experiments so that the 2-μm-tall cells are contained within the evanescent wave at the fiber surface and yield a good IR signal. Once the cells are attached to the fiber, the spectral signature is stable and does not show any sign of cell damage, even after many hours.[15]

To maximize the IR signal, a confluent monolayer of cells at the surface is preferred. A cell coverage analysis was performed on small sections of chalcogenide fibers to characterize the morphology of the cell monolayer.

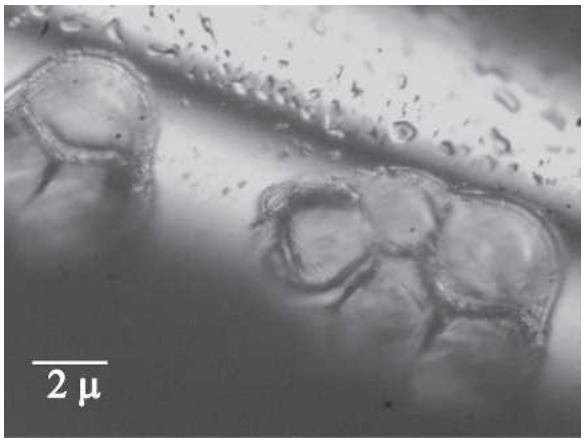

FIG. 1. A549 human lung cells forming strong attachment to the surface of a TAS optical fiber.

Cells on fibers were incubated in darkness and at room temperature for 15 min in a PBS solution with fluorescent dyes. The resulting coverage was evaluated by optical and fluorescence imaging. Figure 2 illustrates the extent of cell coverage on fibers that have been stored in air for several years. It is shown that the cell coverage varies greatly from one experiment to another, resulting in inconsistent and seemingly nonreproducible attachment.

These results suggest that TAS fibers might alter the ability of the cell to form a strong surface attachment, and in particular might exert some negative effect on the cells due to the presence of As in the glass. To investigate a potential toxic behavior, small sections of fiber were immersed in cell cultures and the variation in cellular metabolic activity was probed with a colorimetric assay after 24 h of incubation. This experiment was performed on both old fibers that had been stored in air for 3 years and on fibers washed in water for increasing lengths of time. Finally, the toxicity of the washing solution was also tested.

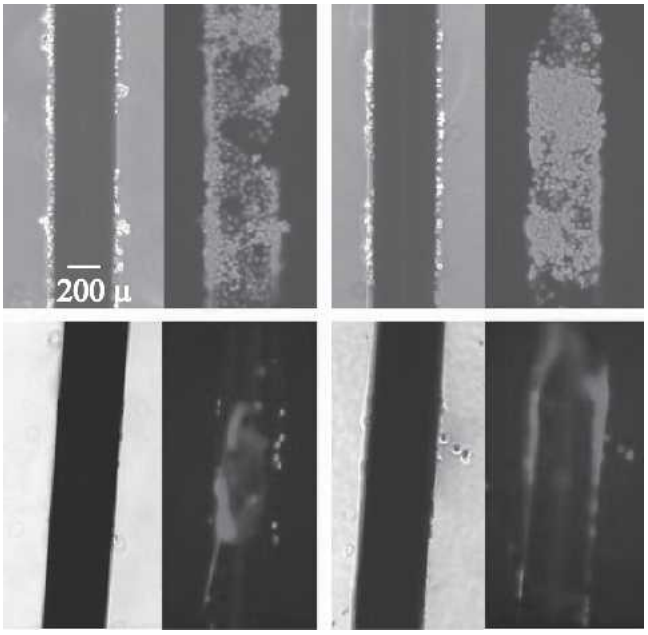

FIG. 2. Optical bright-field images of A549 cell coverage on TAS fibers and the corresponding fluorescence micrograph from the same field of view. Top images show the attachment of confluent monolayers, while the bottom images show poor cell attachment.

The cell-viability data shown in Fig. 3(a) reveal that the 3-year-old fibers exert a significant toxic effect; 35%

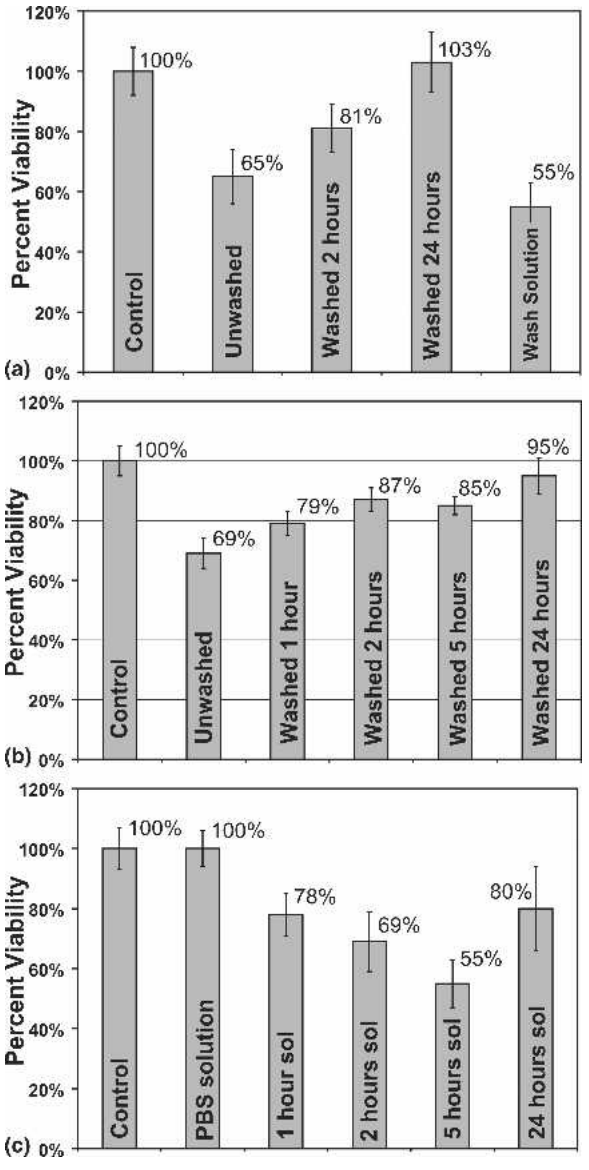

FIG. 3. Viability of cell cultures exposed to sections of TAS chalcogenide fibers as well as solutions in which the fibers have been washed. (a) Viability of A549 human lung cells exposed to a 3-year-old fiber and the same fiber washed in water for 2 h, 24 h, and the resulting washing solution. (b) Viability of rat lung macrophage cells exposed to the same fibers washed in PBS solution for increasing lengths of time. (c) Viability of the same rat lung cells exposed to the PBS washing solution resulting from (b).

of the cells placed in contact with these fibers have died relative to the control. However, when the same experiment is repeated with fibers that have been soaked in water for 2 h, the toxic effect decreases substantially. Finally, fibers washed for 24 h do not exhibit any measurable toxicity. In contrast, the resulting washing solution now shows high toxicity with a 45% decrease in metabolic activity.

This final result clearly indicates that a toxic layer is solubilized when the fibers are immersed in an aqueous solution for periods of time over an hour. Additional experiments performed on rat lung macrophage cells confirm this trend. Figures 3(b) and 3(c) indicate that fibers washed in PBS solution for increasing lengths of time show a continuous decrease in toxicity, while the resulting washing solutions show a corresponding increase in toxicity. In effect, while the toxic layer is etched off the fiber surface, the washing solution becomes enriched with the toxic agent. Here, we must point out an odd data point corresponding to the PBS solution exposed to fibers for 24 h in Fig. 3(c). The toxicity is unexpectedly low (80% viability) in comparison to the high toxicity (55% viability) of the corresponding water-washing solution shown in Fig. 3(a). This result is tentatively explained by suggesting that As might complex with the PBS after 24 h and result in lower effective toxicity to the cells.

Because the observed toxicity is pronounced only for fibers that have been stored in air for long periods of time, it appears likely that the soluble toxic layer is composed of As_2O_3 resulting from a slow reaction with atmospheric oxygen. Indeed, As_2O_3 is highly soluble in water, and the formation of an oxide layer at the fiber surface would therefore be consistent with all the aforementioned observations. To confirm the presence of this oxide layer, a study on the chemical stability of TAS fibers was conducted.

B. Chemical stability of chalcogenide fibers

First, an XPS analysis of the fiber surface was performed to positively establish the presence of an oxide layer. An oxygen atomic concentration of 22.6% was observed at the surface of the old fibers. However, this oxygen content could potentially be associated with the presence of carbonated species at the glass surface. These species are commonly observed by XPS on samples that have been exposed to outside atmosphere. On the other hand, an analysis of the arsenic 3*d* peak can positively establish the presence of oxygen within the glass surface. Figure 4 shows a comparison of the arsenic 3*d* peaks of a freshly drawn fiber (1 week), an old fiber (3 years), a washed fiber, and a reference glass. The reference spectrum was obtained by in situ ion milling of a glass disc sliced from the fiber preform. It can be observed that the old oxidized fiber exhibits a net shift toward higher binding energy, as expected from the formation of an oxide. A similar shift is observed for Te 3*d* and Se 3*d* (data not shown). This clearly suggests that oxygen has indeed reacted with the glass surface to form an oxide layer.

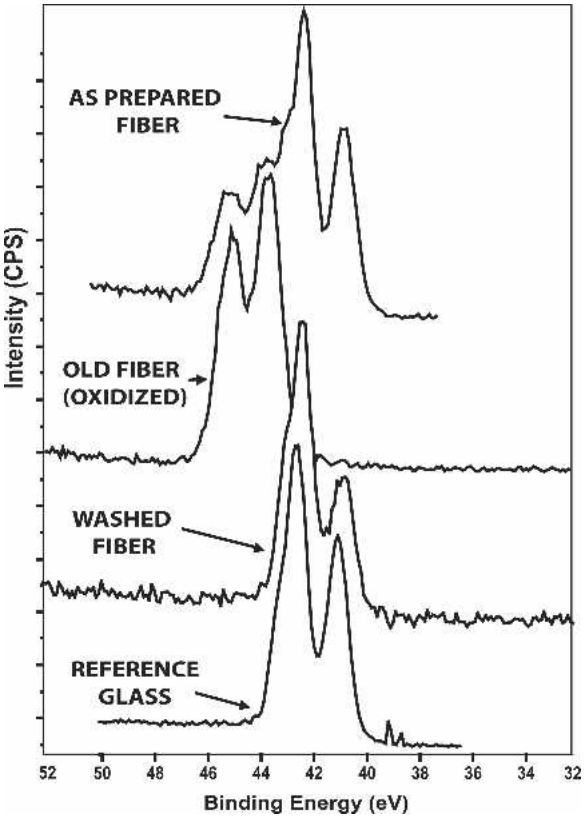

FIG. 4. XPS spectra of a chalcogenide fiber oxidized in air for 3 years, the same washed in water, and a freshly drawn fiber. The spectra are compared to the base glass obtained by in situ milling of a bulk disc of the same composition.

The washed fiber spectrum in Fig. 4 shows that the oxide layer can be entirely removed by soaking the fiber in water. Once the oxide is dissolved, the elemental arsenic is re-exposed and the washed fiber signal appears to be identical to the reference glass signal. Finally, Fig. 4 also reveals that the freshly drawn fiber exhibits a thin oxide layer that is estimated to be only a few angstroms thick. The XPS spectrum was collected about a week after fiber drawing, and it is not clear whether this slight surface oxidation is intrinsic to the drawing process or took place during the days following it.

To further confirm the oxide dissolution process and study its kinetics, an analysis of the washing solution was performed using ICP-MS. Old fibers as well as previously washed fibers were soaked in saline solution for up to 48 h, and the concentration of arsenic etched out into the solution was measured as a function of time. Figure 5 shows that the oxide dissolution is complete after 4 h. After that time, the arsenic concentration reaches a plateau and stays constant. Similarly, a washed fiber shows

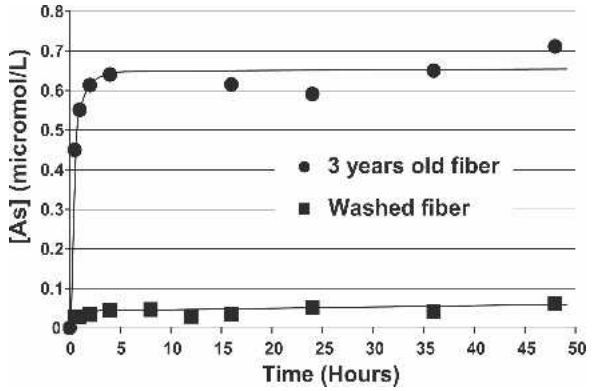

FIG. 5. Variation of the total arsenic concentration with dissolution time for an oxidized fiber and a washed fiber immersed in saline solution. The arsenic release is measured by ICP-MS. The arsenic in solution is both in the As^{III} and As^{V} form.

a constant and barely detectable amount of arsenic that has leached into the solution. These results confirm that once the oxide layer has been removed the chalcogenide glass itself is essentially stable in aqueous solution and does not dissolve to a measurable extent within a few days. This result therefore suggests that these chalcogenide fibers can be safely used for optical probing in aqueous environments without significant damage to the fiber.

Finally, the extent of surface damage was investigated by monitoring the change in surface topology following dissolution. Figure 6 shows an AFM image of a 3-year-old fiber before and after soaking in water for 24 h. The surface roughness visibly increases after the dissolution of the oxide layer. The oxide layer formation appears to be fairly nonuniform at the microscopic level and results in the formation of pits on dissolution. These features are presented quantitatively in Fig. 7, which shows the topology profile along several randomly chosen lines across the samples. The old fiber has a relatively smooth surface, while the etched fiber contains pits with a depth

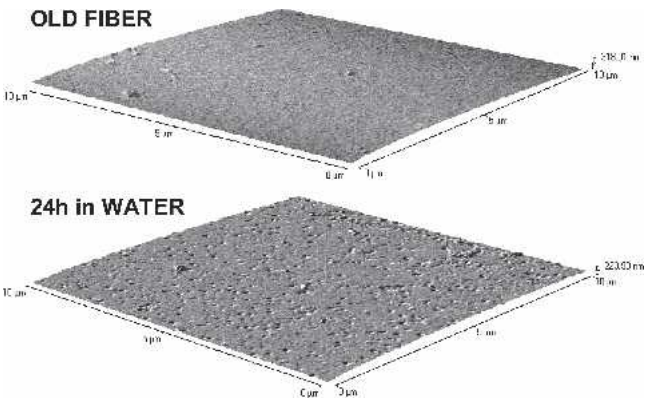

FIG. 6. Two-dimensional AFM image of the surface of a fiber oxidized in air for 3 years, and the same fiber after immersion in an aqueous solution for 24 h.

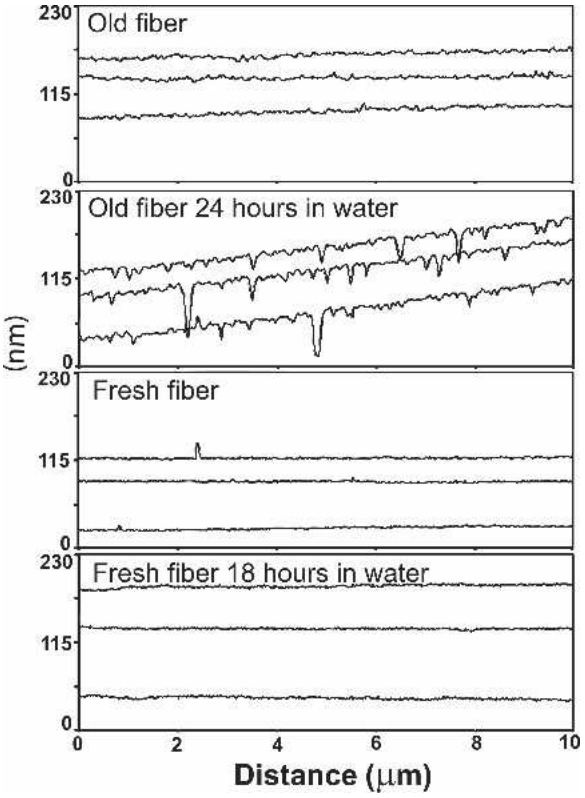

FIG. 7. One-dimensional surface profile of the fiber shown in Fig. 6 as well as a new fiber before and after immersion in water for 18 h. The line profiles are obtained by taking three random lines across the topological map shown in Fig. 6.

of up to 50 nm. On the other hand, the fresh fiber does not show significant surface changes even after 18 h in water. This is consistent with the previous ICP-MS results, which suggest that the glass in its nonoxidized form is resistant to dissolution in water.

IV. DISCUSSION

Both the toxicity and chemical stability results presented above are consistent with the formation of an oxide layer on the surface of TAS fibers. The XPS spectra clearly show that oxygen is incorporated within the glass network to form a mixed oxide of As, Te, and Se. The oxidation process, however, is fairly slow, and an estimate based on the density of TAS glass and the amount of dissolved arsenic measured by ICP-MS shows that the oxide layer is only 5 nm thick after 3 years of exposure to atmospheric oxygen (and possibly atmospheric water vapor). Unlike the TAS glass itself, this oxide layer is highly soluble in aqueous solution and can be easily removed by washing. The resulting roughness measured by AFM suggests that this layer is highly irregular. While the overall amount of oxide removed is equivalent to a 5-nm-thick layer, pit formation as deep as 50 nm can be observed.

The dissolution of an arsenic-based oxide is of concern for application in cell-based sensors. Even though these oxide layers are fairly thin, the amount of arsenic released can cause problems if old fibers are put into direct contact with live cells. A decrease in cell attachment and metabolic activity can result from exposure to a highly oxidized fiber. However, the good correlation between the etching time and the vanishing of toxicity suggests that simple washing can resolve this issue. Alternatively, the use of fresh fibers is also an option to prevent toxic effects. Figure 4 shows a small extent of oxidation even on fresh fibers, but, considering that the penetration depth of our XPS is about 30 Å, it is estimated that the surface oxidation on a new fiber is <10 Å (i.e., a few atomic layers). Such a thin layer can be rapidly dissolved when in contact with an aqueous solution and a 5-cm fiber could be dissolved to a safe As level within less than a tenth of a cubic centimeter of water. This is consistent with the finding that lung cell cultures consistently formed attachments to new fibers immersed in 0.9% saline solutions.[15] The thin oxide layer is quickly dissolved in the saline solution, and the diffusion of the highly toxic and mobile arsenate ions within the cells is prevented. The elemental arsenic within the glass network, on the other hand, is quite stable and insoluble, as suggested by the absence of toxicity observed in Fig. 3(a). The cells can then display a normal level of metabolic activity when attached to a clean TAS glass surface. Indeed, when fresh fibers are used in aqueous solution, the coverage and optical signal are consistently good.

Consequently, live cells can be safely attached to a TAS glass surface and used for biosensing applications. As shown previously,[15] the spectral features of cells on TAS glass are stable, while they show strong spectral response on exposure to a toxicant. Hence, these cells can be reliably used to sense the presence of environmental toxicants without interference from the glass substrate.

V. CONCLUSIONS

Amorphous chalcogenide materials have recently gained interest for IR-based biosensing applications. Several of these glass formulations are stabilized with As and are now becoming commercially available. These glasses form an oxidation layer of a few nanometers on long-term storage in air. It is shown that chalcogenide glass containing arsenic can exhibit a toxic effect, after significant oxidation, due to the dissolution of the oxide layer in aqueous environments. However, this oxide layer can be easily washed away in small amounts of water, and the original glass in the nonoxidized form is reexposed at the fiber surface. During conventional measurements of up to a couple of days in solution, the original glass exhibits good stability in water and has no measurable toxic effects. These results are consistent with previous findings, which show that fresh fibers can be safely used for biosensing experiments involving direct contact with live human lung cells.

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REFERENCES

1. B. Bureau, X.H. Zhang, F. Smectala, J.L. Adam, J. Troles, H.L. Ma, C. Boussard-Pledel, J. Lucas, P. Lucas, D. Le Coq, M.R. Riley, and J.H. Simmons: Recent advances in chacogenide glasses. *J. Non-Cryst. Solids* **345,** 276 (2004).
2. J.S. Sanghera, L.B. Shaw, L.E. Busse, V.Q. Nguyen, P.C. Pureza, B.C. Cole, B.B. Harbison, I.D. Aggarwal, R. Mossadegh, F. Kung, D. Talley, D. Rossel, and R. Miklos: Development and infrared applications of chalcogenide glass optical fibers. *Fiber Int. Optics* **19,** 251 (2000).
3. G. Lund and H. Bonnet: DARWIN: The infrared space interferometer. *Comptes Rendus Acad. Sci.* **2,** 137 (2001).
4. J.S. Sanghera, L.B. Shaw, L.E. Busse, D. Talley, and I.D. Aggarwal: Infrared transmitting fiber optics for biomedical applications. Proceedings Photonics West, San Diego, CA, *SPIE* **3596,** 178 (1999).
5. H. Steiner, M. Jakusch, M. Kraft, M. Karlowatz, T. Baumann, R. Niessner, W. Konz, A. Brandenburg, K. Michel, C. Boussard-Pledel, B. Bureau, J. Lucas, Y. Reichlin, A. Katzir, N. Fleischmann, K. Staubmann, R. Allabashi, J.M. Bayona, and B. Mizaikoff: In situ sensing of volatile organic compounds in groundwater: First field tests of a mid-infrared fiber-optic sensing system. *Appl. Spectrosc.* **57,** 607 (2003).
6. P. Lucas, M.R. Riley, C. Boussard-Pledel, and B. Bureau: Advances in chalcogenide fiber evanescent-wave biochemical sensing. *Anal. Biochem.* **351,** 1 (2006).
7. S. Hocde, O. Loreal, O. Sire, C. Boussard-Pledel, B. Bureau, B. Turlin, J. Keirsse, P. Leroyer, and J. Lucas: Metabolic imaging of tissues by infrared fiber-optic spectroscopy: An efficient tool for medical diagnosis. *J. Biomed. Opt.* **9,** 404 (2004).
8. K. Michel, B. Bureau, C. Boussard-Pledel, T. Jouan, J.L. Adam, K. Staubmann, and T. Baumann: Monitoring of pollutant in wastewater by infrared spectroscopy using chalcogenide glass optical fibers. *Sens. Actuators, B* **101,** 252 (2004).
9. J.S. Sanghera, I.D. Aggarwal, L.E. Busse, P.C. Pureza, V.Q. Nguyen, F.H. Kung, L.B. Shaw, and F. Chenard: Chalcogenide optical fibers target mid-IR applications. *Laser Focus World* **41,** 83 (2005).
10. R. Krska, E. Rosenber, K. Taga, R. Kellner, A. Messica, and A. Katzir: Polymer coated silver halide infrared fibers as sensing devices for chlorinated hydrocarbons in water. *Appl. Phys. Lett.* **61,** 1778 (1992).

11. M. Karlowatz, M. Kraft, and B. Mizaikoff: Simultaneous quantitative determination of benzene, toluene, and xylenes in water using mid-infrared evanescent field. *Anal. Chem.* **76,** 2643 (2004).
12. K. Taga, R. Kellner, U. Kainz, and U.B. Sleytr: In situ attenuated total reflectance ft-ir analysis of an enzyme-modified mid-infrared fiber surface using crystalline bacterial surface proteins. *Anal. Chem.* **66,** 35 (1994).
13. B. Mizaikoff, R. Gobel, R. Krska, K. Taga, R. Kellner, M. Tacke, and A. Katzir: Infrared fiber-optical chemical sensors with reactive surface coatings. *Sens. Actuators, B* **29,** 58 (1995).
14. C.X. Yu, A. Ganjoo, H. Jain, C.G. Pantano, and J. Irudayaraj: Mid-IR biosensor: Detection and fingerprinting of pathogens on gold island functionalized chalcogenide films. *Anal. Chem.* **78,** 2500 (2006).
15. P. Lucas, D. Le Coq, C. Juncker, J. Collier, D.E. Boesewetter, C. Boussard-Plédel, B. Bureau, and M.R. Riley: Evaluation of toxic agent effects on lung cells by fiber evanescent wave spectroscopy (FEWS). *Appl. Spectrosc.* **59,** 1 (2005).
16. P. Lucas, M.A. Solis, D. Le Coq, C. Juncker, M.R. Riley, J. Collier, D.E. Boeswetter, C. Boussard-Pledel, and B. Bureau: Infrared biosensors using hydrophobic chalcogenide fibers sensitized with live cells. *Sens. Actuators, B* **119,** 355 (2006).
17. M.R. Riley, D.L. DeRosa, J. Blaine, B.G. Potter, P. Lucas, D. Le Coq, C. Juncker, D.E. Boeswetter, J. Collier, C. Boussard-Pledel, and B. Bureau: Biologically inspired sensing: Infrared spectroscopic analysis of cell responses to an inhalation health hazard. *Biotechnol. Prog.* **22,** 24 (2006).
18. M.R. Riley, I.M. Fernandez, and P. Lucas: Spectroscopic analysis of cell physiology and function, in *Frontiers in Drug Design and Discovery 256,* edited by G.W. Caldwell, Atta-ur-Rahman, M.R. D'Andrea, and M.I. Choudhary (Bentham Science Publishers Ltd., Pennington, NJ, 2006).
19. Y. Fang, A.M. Ferrie, N.H. Fontaine, J. Mauro, and J. Balakrishnan: Resonant waveguide grating biosensor for living cell sensing. *Biophys. J.* **91,** 1925 (2006).
20. D.A. Stenger, G.W. Gross, E.W. Keefer, K.M. Shaffer, J.D. Andreadis, W. Ma, and J.J. Pancrazio: Detection of physiologically active compounds using cell-based biosensors. *Trends Biotechnol.* **19,** 304 (2001).
21. B.D. DeBusschere and G.T.A. Kovacs: Portable cell-based biosensor system using integrated CMOS cell-cartridges. *Biosens. Bioelectron.* **16,** 543 (2001).
22. W. Yicong, W. Ping, Y. Xuesong, Z. Gaoyan, H. Huiqi, Y. Weimin, Z. Xiaoxiang, H. Jinghong, and C. Dafu: Drug evaluations using a novel microphysiometer based on cell-based biosensors. *Sens. Actuators, B* **80,** 215 (2001).
23. Y. Gotshal, R. Simhi, B-A. Sela, and A. Katzir: Blood diagnostic using fiberoptic evanescent wave spectroscopy and neural networks analysis. *Sens. Actuators, B* **42,** 157 (1997).
24. O. Eytan, B-A. Sela, and A. Katzir: Fiber-optic evanescent-wave spectroscopy and neural network: Application to chemical blood analysis. *Appl. Opt.* **39,** 3357 (2000).
25. J. Keirsse, C. Bousard-Pledel, O. Loreal, O. Sire, B. Bureau, P. Leroyer, B. Turlin, and J. Lucas: IR optical fiber sensor for biomedical applications. *Vib. Spectrosc.* **32,** 23 (2003).
26. D. Le Coq, K. Michel, J. Keirsse, C. Boussard-Pledel, G. Fonteneau, B. Bureau, J-M. Le Quere, O. Sire, and J. Lucas: Infrared glass fibers for in-situ sensing, chemical and biochemical reactions. *C. R. Chimie* **5,** 907 (2002).
27. D. Beyersmann: Effects of carcinogenic metals on gene expression. *Toxicol. Lett.* **127,** 63 (2002).
28. S. Hocde, C. Boussard-Pledel, G. Fonteneau, and J. Lucas: Chalcogens based glasses for IR fiber chemical sensors. *Solid State Sci.* **3,** 279 (2001).
29. V.S. Shiryaev, J-L. Adam, X.H. Zhang, C. Boussard Pledel, J. Lucas, and M.F. Churbanov: Infrared fibers based on Te–As–Se system with low optical losses. *J. Non-Cryst. Solids* **336,** 113 (2004).
30. D. Lecoq, K. Michel, G. Fonteneau, S. Hocde, C. Boussard-Pledel, and J. Lucas: Infrared chalcogen glasses: Chemical polishing and fiber remote spectroscopy. *Int. J. Inorg. Mater.* **3,** 233 (2001).
31. C.D. Okeson, M.R. Riley, and E. Riley-Saxton: In-vitro alveolar cytotoxicity of soluble components of airborne particulate matter: Effects of serum on toxicity of transition metals. *Toxicol. in Vitro* **18,** 673 (2004).