Comparison of neuronal cell adhesiveness of materials in the diX (Parylene) family

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ABSTRACT

DiX C (or Parylene-C) has been widely used as a coating material to insulate neural electrodes in recent decades. However, its uses are limited due to its extremely low adhesiveness with neuronal cells. Other functional materials in the diX family, such as diX A, diX AM, and diX H, have been commercialized recently and would offer different features in biocompatibility from diX C. However, their cell adhesiveness remains unknown. In this work, we used an *in vitro* approach to investigate how the surface of each material in the diX family affects the degree of neuronal cell adhesiveness compared with a conventional culture dish of polystyrene (PS). The neuronal cell adhesiveness on diX AM and diX H was almost equivalent to that for the PS dish, whereas neuronal cells did not settle on the surface of diX C and diX A. Our results suggest that diX AM and diX H could provide another practical feature as a coating material for a scaffold in a substrate with any configuration in neural devices.

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Poly(monochloro-para-xylene), commercially called diX C or Parylene-C, has been widely used as a coating to insulate neural electrodes [8–11,13,16]. This is because chemical vapor deposition of diX C provides a thin, conformal, and pinhole-free coating on virtually any substrate, which protects substrates from moisture, chemicals, and electric charge [4,10,11] and is also compatible with a micromachining process [8–10,14,17]. However, the usage of diX C is limited to neural electrodes. It cannot be applied as a coating material for other neural devices, such as regeneration-type electrodes for interfacing with the peripheral nervous system and multi-electrode array systems for *in vitro* electrophysiology. A coating material for such neural devices must be able to promote cellular growth and repopulation for stable recording and stimulation. However, diX C cannot support and promote the growth of neuronal cells because its hydrophobic characteristic leads to extremely low adhesiveness with them. Thus, diX C is not suitable as a scaffold for the regeneration and extracellular matrix of neuronal cells.

Recently, other functional materials in the diX family, such as diX A, diX AM, diX H, diX SR, diX D, and diX CF, have become commercially available. These materials have desirable physical characteristics similar to those of diX C, such as excellent gas-barrier and mechanical properties, and, like diX C, can be coated by means of chemical vapor deposition. More importantly, they may offer functionalized features that diX C does not. In particular, three materials – diX A [poly(monoamino-para-xylene)], diX AM [poly(monoaminomethyl-para-xylene)], and diX H [poly(monoaldehyde-para-xylene)] – could offer improved biocompatibility. This is because diX A and diX AM promote bioactivity by providing an amino group ($-NH_2$) and because diX H contains an aldehyde group ($-CHO$) for immobilization of biomolecules (see Fig. 1 for their chemical structures). However, despite the apparent physical properties of these materials, their biocompatibility remains unknown. In this work, using neuron-like cells, i.e., the rat adrenal pheochromocytoma cell line (PC12 cells), we investigated their biocompatibility, particularly how their surfaces affect the degree of neuronal cell morphology and adhesiveness compared with the conventional culture dish of polystyrene (PS).

Ten-centimeter-diameter PS culture dishes (3020-100, IWAKI) were coated with diX A, diX AM, diX C or diX H (Daisankasei Co., Ltd.). The coating method was the same as that for diX C and has been described elsewhere [8–10,14]. PC12 cells (RCB-0009, Riken

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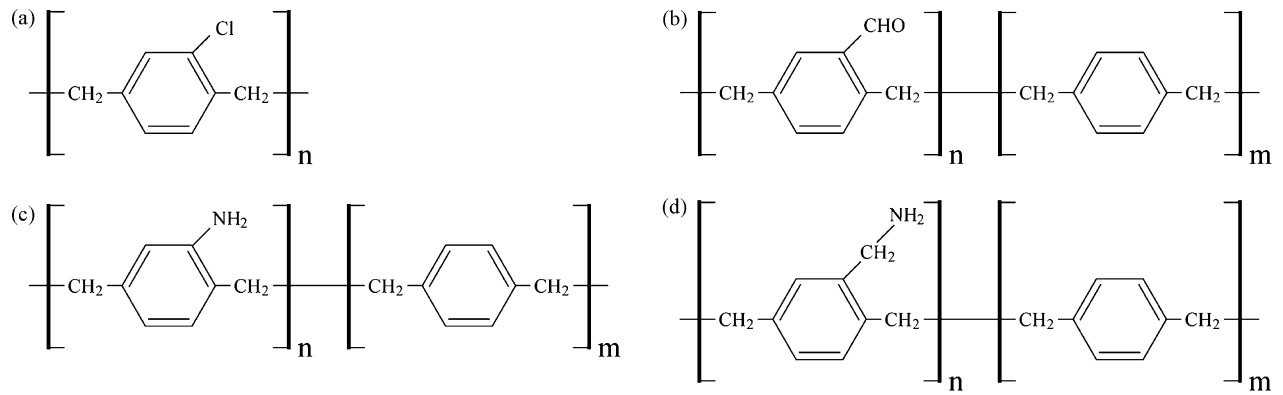
Fig. 1. Chemical structures of (a) diX C [poly(monochloro-para-xylene)], (b) diX H [poly(monoaldehyde-para-xylene)], (c) diX A [poly(monoamino-para-xylene)], and (d) diX AM [poly(monoaminomethyl-para-xylene)].

Cell Bank), which serve as a standard model for neuronal cells [1,7,15], were maintained in a neuro basal medium (21103, GIBCO) containing 5% horse serum and 10% fetal bovine serum at 37 °C in humidified atmosphere containing 5% CO_2. PC12 cells were cul-

tured on each dish for 144 h. Their morphologies were observed with an inverse phase contrast microscope (IX-70, Olympus), and more than seven pictures for each type of a dish were analyzed to manually count the numbers of cells adhering to each surface at 72
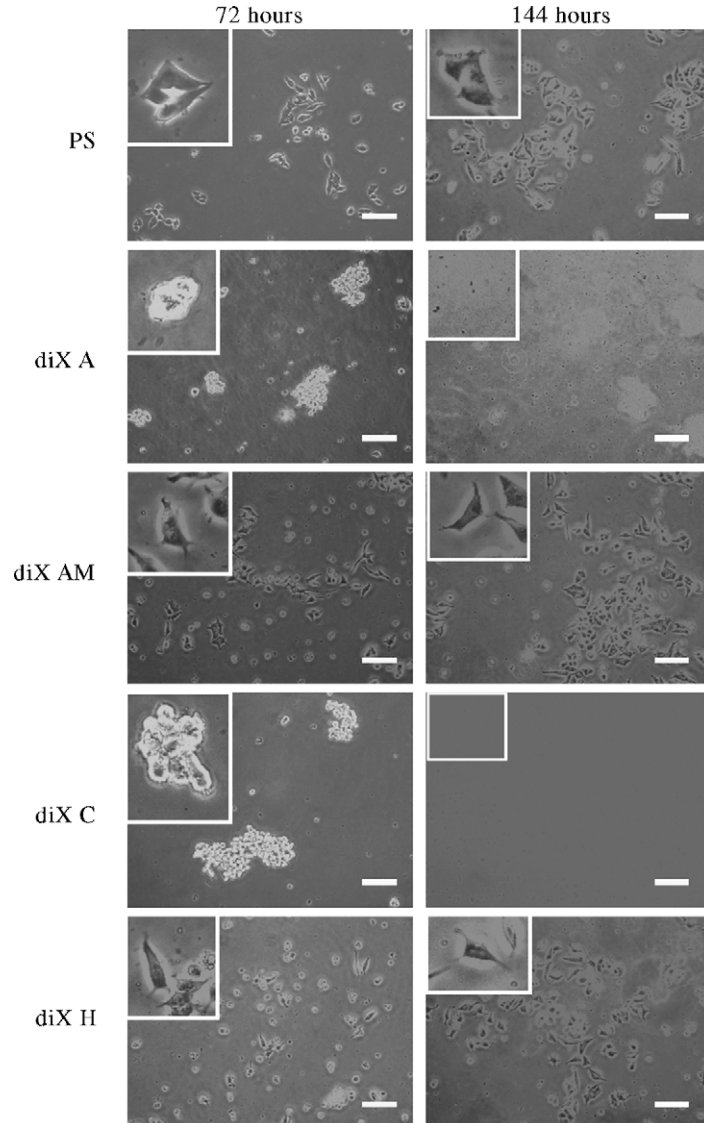
Fig. 2. Morphologies of PC12 cells on PS, diX A, diX AM, diX C, and diX H at 72 h (left column) and 144 h (right column). Scale bar is 100 μm. Each top-left image is a 4× magnification.
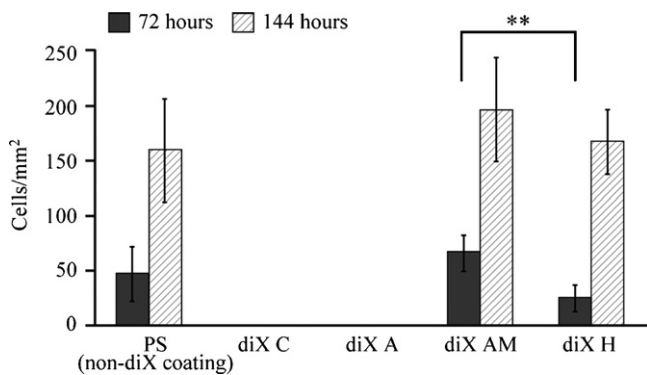
Fig. 3. Adhesiveness of PC12 cells on the surface of materials in the diX family and PS (Student's *t*-test; ** $p < 0.01$). Error bars show the standard deviations.

and 144 h. The control dish was a standard PS culture dish (3020-100, IWAKI) without any diX-based materials coated on a substrate [3].

Fig. 2 shows the PC12 cell morphologies for all specimens at 72 and 144 h. The specimens reached confluence at 144 h. DiX A and diX C had extremely low adhesiveness to PC12 cells in all periods of culturing. PC12 cells did not adhere on these surfaces. Instead, they floated in the culturing medium or a few cells settled on the surface with a rounded shape. In contrast, PC12 cells adhered to the surface of the diX AM and diX H and showed obvious outgrowth of neurites. Notably, there were no differences in the morphologies of the PC12 cells that had adhered to the non-diX-coated PS of the standard culture dish in all periods.

Fig. 3 shows the average density of adhering PC12 cells for all specimens. The error bars show the standard deviations of the means in images. Significantly larger numbers of cells adhered to the surface of diX AM and diX H ($p < 0.05$ with Tukey–Kramer test) than to the surface of diX A and diX C, whereas hardly any PC12 cells were observed on diX A and diX C even at 144 h. The number of cells adhering to the surface of diX AM and diX H was comparable to the number adhering to the standard culture dish of PS in all periods ($p > 0.1$ with Tukey–Kramer test). The number of adhering cells between diX AM and diX H was different at 72 h ($p < 0.01$ with Student's *t*-test), but there was no significant difference at 144 h ($p > 0.1$ with Student's *t*-test).

The amino group on the surface of a substrate is thought to promote adhesiveness and the growth of neurons [13], suggesting that both diX A and diX AM could promote cellular outgrowth. However, our results showed that PC12 cells adhered to diX AM. This is probably because diX AM has a spacer of methylene between the benzene ring and amino group, whereas diX A does not and therefore directly couples between the benzene ring and amino group. Many groups have reported that protein adhesiveness increases with a spacer [5], implying the promotion of cell adhesiveness [6,16]. This supports our finding that diX AM has considerably better adhesiveness than diX A.

Cell adhesiveness to diX H was not high at 72 h, but it increased till 144 h and reached confluence to become almost equivalent to that of the PS dishes. An aldehyde group is known to conjugate amines on proteins [12]. This means that the aldehyde group of diX H would improve adhesiveness to neuronal cells such as seen in our results.

Recently, Chang et al. reported that changing the diX C surface from hydrophobic to hydrophilic with an oxygen plasma treatment improved cell adhesiveness [2]. This method is applicable, but there is a technical drawback because the face exposed to plasma treatment can only have a two-dimensional structure. On the other hand, diX AM or diX H can be coated on a substrate with any configuration and the coating can be achieved with the same conventional coating process used for diX C without any extra process steps.

In conclusion, this study examined neuronal cell adhesiveness on the surface of materials in the diX family. Although the adhesion of diX A was almost equivalent to that of diX C, diX AM and diX H achieved high cellular adhesiveness close to that of the standard PS tissue culture. Thus, diX AM and diX H potentially promise another practical feature as a coating material for a scaffold in a substrate with any configuration in neural devices.

References

[1] D.G. Attiah, R.A. Kopher, T.A. Desai, Characterization of PC12 cell proliferation and differentiation-stimulated by ECM adhesion proteins and neurotrophic factors, J. Mater. Sci. Mater. Med. 14 (2003) 1005–1009.

[2] T.Y. Chang, V.G. Yadav, S. De Leo, A. Mohedas, B. Rajalingam, C.L. Chen, S. Selvarasah, M.R. Dokmeci, A. Khademhosseini, Cell and protein compatibility of Parylene-C surfaces, Langmuir 23 (2007) 11718–11725.

[3] A. Curtis, J.V. Forrester, C. McInnes, F. Lawrie, Adhesion of cells to polystyrene surfaces, J. Cell. Biol. 97 (1983) 1500–1506.

[4] M. Fujihira, M. Fukui, T. Osa, Chemically modified parylene gate field effect transistors, preparation of pH insensitive Parylene gate for chemical modification, J. Electroanal. Chem. 106 (1980) 413–418.

[5] K. Fujita, N. Nakamura, H. Ohno, B.S. Leigh, K. Niki, H.B. Gray, J.H. Richards, Mimicking protein-protein electron transfer: voltammetry of *Pseudomonas aeruginosa* azurin and the *Thermus thermophilus* Cu(A) domain at omega-derivatized self-assembled-monolayer gold electrodes, J. Am. Chem. Soc. (2004) 13954–13961.

[6] A. Gessner, A. Lieske, B.R. Paulke, R.H. Müller, Functional groups on polystyrene model nanoparticles: influence on protein adsorption, J. Biomed. Mater. Res. A 65 (2003) 319–326.

[7] L.A. Greene, A.S. Tischler, Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells with respond to nerve growth factor, Proc. Natl. Acad. Sci. U.S.A. 73 (1976) 2424–2428.

[8] J.M. Hsu, L. Rieth, R.A. Normann, P. Tathireddy, F. Solzbacher, Encapsulation of an integrated neural interface device with Parylene C, IEEE Trans. Biomed. Eng. 56 (2009) 23–29.

[9] Y. Kato, I. Saito, T. Hoshino, T. Suzuki, K. Mabuchi, Preliminary study of multichannel flexible neural probes coated with hybrid biodegradable polymer, Proc. IEEE EMBS (2006) 660–663.

[10] G.E. Loeb, M.J. Bak, M. Salcman, E.M. Schmidt, Parylene as a chronically stable, reproducible microelectrode insulator, IEEE Trans. Biomed. Eng. 24 (1977) 121–128.

[11] G.E. Loeb, A.E. Walker, S. Uematsu, B.W. Konigsmark, Histological reaction to various conductive and dielectric films chronically implanted in the subdural space, J. Biomed. Mater. Res. 11 (1977) 195–210.

[12] G. MacBeath, S.L. Schreiber, Printing proteins as microarrays for high-throughput function determination, Science 289 (2000) 1760–1763.

[13] Y. Naka, A. Eda, H. Takei, N. Shimizu, Neurite outgrowths of neurons on patterned self-assembled monolayers, J. Biosci. Bioeng. 94 (2002) 434–439.

[14] X. Navarro, T.B. Krueger, N. Lago, S. Micera, T. Stieglitz, P. Dario, A critical review of interfaces with the peripheral nervous system for the control of neuroprostheses and hybrid bionic systems, J. Peripher. Nerv. Syst. 10 (2005) 229–258.

[15] M. Sano, K. Kato, T. Totsuka, R. Katoh-Semba, A convenient bioassay for NGF using a new subline of PC12 pheochromocytoma cells (PC12D), Brain Res. 459 (1988) 404–406.

[16] D. Tebbe, R. Thull, U. Gbureck, Influence of spacer length on heparin coupling efficiency and fibrinogen adsorption of modified titanium surfaces, Biomed. Eng. Online 6 (2007).

[17] S. Takeuchi, D. Ziegler, Y. Yoshida, K. Mabuchi, T. Suzuki, Parylene flexible neural probes integrated with microfluidic channels, Lab. Chip 5 (2005) 519–523.