# Cytotoxicity of Platinum Black

**Schuettler M[1], Doerge T[2], Wien SL[2], Becker S[2], Staiger A[3], Hanauer M[2], Kammer S[2], Stieglitz T[4]**

[1] University College London, Implanted Devices Group, Malet Place Engineering Building, Gower Street, London WC1E 6BT, United Kingdom
[2] Fraunhofer-Institute for Biomedical Engineering, Ensheimer Str. 48, D-66386 Sankt Ingbert, Germany
[3] Micromotion GmbH, An der Fahrt 13, D-55124 Mainz, Germany
[4] Institute for Microsystem Technology (IMTEK), Laboratory for Biomedical Microtechnology, University of Freiburg, Georges-Koehler-Allee 102, D-79110 Freiburg, Germany

schuettler@ieee.org

## Abstract

***Platinum black is a coating material for neural electrodes which has the potential to lower the phase-boundary impedance of the electrode-tissue interface dramatically. Depositing platinum black by means of electroplating involves the use of the toxic heavy metal lead as an electrolyte ingredient. Using surface analytical methods, we could not exclude the possibility that traces of lead were integrated into the actual coating. Cytotoxicity tests showed that one of two tested cell lines were affected when exposed to a platinum black extract: The DNA synthesis was disturbed for one fifth of all exposed OLN-93 rat oligodendrocytes. The metabolism of the cells was not affected. However, mouse fibroblasts L-929 did not show any adverse reaction to platinum black. As a conclusion we recommend the use of platinum black as electrode coating for most animal experiments but not for chronic human implants.***

## 1. INTRODUCTION

Some neuroprosthetic applications, especially those which restore senses like vision or hearing, require large numbers of electrode contacts arranged on a very restricted space. High integration densities involve small electrode contact sizes, which are of high impedance. The higher the impedance the higher the electrical losses across the phase boundary which is hindering for neural stimulation and for recording. Different sorts of electrode coatings like TiN and IrOx have been proposed to overcome this problem [1]. A third, commonly used coating, is platinum black. It is basically sponge-like platinum, which has, compared to platinum foil or sputtered platinum films, a very large effective surface area. The larger effective surface area causes a drop in electrode impedance, potentially by some orders of magnitude [2]. A simple method to coat electrodes with platinum black is electroplating. In order to obtain the sponge-like character, an electrolyte is used that contains the toxic heavy metal lead. In this study, we investigate whether the lead is actually deposited on the targeted surface and if the coating has toxic properties that affect metabolism and/or proliferation of cells.

## 2. METHODS

### 2.1 Depositing Platinum Black

Micromachined polyimide electrode structures were used to deposit platinum black on. These structures consisted of a 300 nm gold layer, sandwiched between a bottom and a top layer of 5 µm polyimide (details of fabrication process: see [3]). Each fabricated structure had a geometrical area of 100 $mm^2$. The top layer was partly removed, so that a total area of 50 $mm^2$ of the gold metal film was exposed, onto which platinum black was electrochemically deposited from an electrolyte (the relatively large electrode area was chosen to ease the cytotoxicity tests). The electrolyte was produced by dissolving 5 g $H_2PtCl_6$ in 357 ml ultra pure water and by adding 71.4 mg $Pb(NO_3)_2$ (Chemicals: Merck KGaA, Darmstadt, Germany). Voltage-controlled electroplating was carried out, using a platinum counter electrode (anode) and an Ag/AgCl reference electrode, connected to a potentiostat. 250 $mV_{DC}$ was applied to the electrolyte for a time periode of 10 s while the starting layer of electrode structure was kept at ground potential (cathode). During the deposition process, ultrasound was introduced into the electrolyte in order to blast off poorly adhesive platinum black particles from the electrode structure. After electroplating, the coated surfaces were rinsed in ultra pure water for two hours.

### 2.2 Material Analysis of Platinum Black

Energy dispersive x-ray (EDX) surface analysis scans were performed on two samples: A) Deposited platinum black and B) a drop of electrolyte (dried). The results from the scans were material specific profiles over an energy spectrum. Chemical elements are represented by specific lines in the energy spectrum.

### 2.3 Investigating Cytotoxic Properties of Platinum Black

Cell toxicity tests were carried out according to the European Standards ISO 10993-5 and ISO 10993-12. Two different cell lines were used: OLN-93 (rat oligodendrocyte) and L-929 (mouse fibroblasts). Reaction to direct contact and to extracts were investigated as follows:
1.) The cells were left for 48 h in direct contact to the platinum black surface. Subsequently, a dye was applied to indicate cell viability. The ratio of dead to viable cells was determined and used to rank the reactivity of platinum black in a scale from "no reactivity" to "very reactive" (grades 0 and 4, respectively).
2.) An extract from the platinum black coated samples was obtained by incubating them for 24 hours in cell culture medium. Into the so obtained extract, which contained soluble agents from the samples, cells were injected and incubated for 24 hours. Subsequently it was determined to what extent the metabolic activity (WST-1 test[1]) and the DNA synthesis (BrdU test[2]) of the cells were affected.
Toxic effects on cells were quantitatively detected by monitoring the extinction of the reaction products according to the WST-1/BrdU assay. After subtraction of the natural extinction rate, the results for each sample were calculated by statistical means. Measurements with control cultures[3] that were incubated with cell culture medium, determined the 100 % rate.

## 3. RESULTS

### 3.1 Appearance of Platinum Black Coating

The applied electroplating parameters led to a uniform layer of platinum black on top of the sputtered gold contacts. The layer thickness was estimated using scanning electron microscopy (SEM) to be about 2-3 µm in thickness. The SEM pictures (Figure 1) show the rough, sponge-like structure of platinum black. Visually inspected, platinum black appeared as a uniform dark black layer.

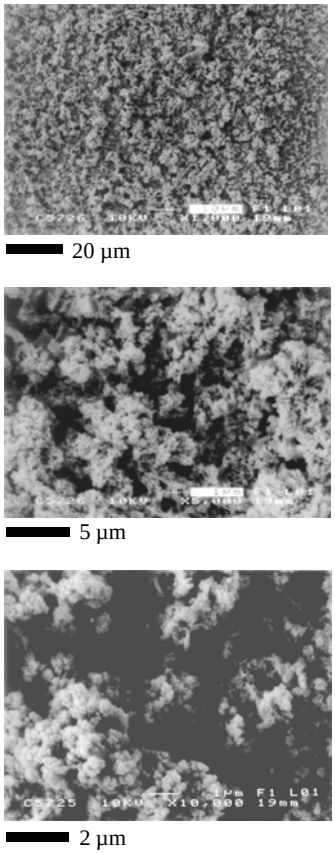


Figure 1: SEM pictures of platinum black at different resolutions show its sponge-like character.

### 3.2 Material Composition of Platinum Black

Comparing the profiles from the EDX scans of electrolyte and actual deposited platinum black coating (Figure 2), reveals two major differences between them:
1.) The concentration of chlorine in the electrolyte sample was very prominent (spectral lines at about 2.65 keV and 2.8 keV). Hardly any chlorine was detected in the platinum black coating.
2.) Lead was detected in the electrolyte at 1.85 keV and possibly at 2.35 keV (the later count could also be caused by platinum, having a spectral line at a very similar energy). We did not find a peak in the deposited platinum black

[1] Tetrazolium salt "WST-1" (Roche Diagnostics, Mannheim, Germany) is reduced enzymatically and formazan is produced.

[2] DNA synthesis was measured by the insertion of the base analogue 5-Bromo-2'-desoxyuridin "BrdU" (BrdU; cell proliferation ELISA BrdU (colorimetric), Roche Diagnostics).

[3] Negative reference: Polypropylen tubing (RAU-PP 1463, Rehau AG & CO, Rehau, Germany). Positive reference: (2-Hydroxethyl)-methacrylat "HEMA" (Merck KGaA).

layer at 1.85 keV and the peak at 2.35 keV can be interpreted as a reference to lead as well as to platinum. References to platinum were also found at higher energies, such as 9.2 keV, 11.1 keV and 11.3 keV (platinum black sample). The high energy reference to lead (10.55 keV) was not represented in the scanned profile.

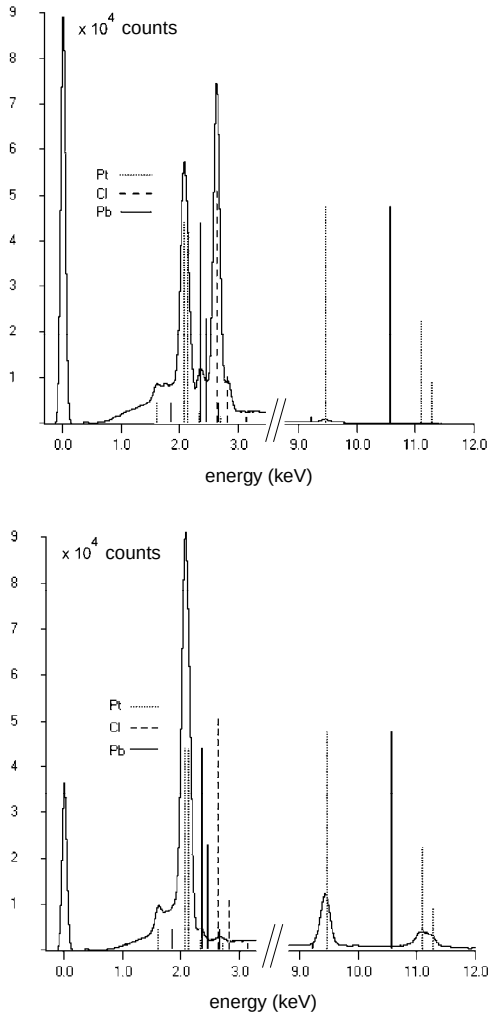


Figure 2: EDX-Scans of electrolyte (top) and deposited platinum black (bottom).

### 3.3 Cytotoxicity of Platinum Black

Direct contact tests showed "no reactivity" (grade 0) of platinum black coatings for both cell lines. Extract tests gave different results for the two cell lines (Tables 1 and 2: mean values and their standard deviation (SD)). While the mouse fibroblasts L-929 were affected neither in their proliferation nor in the metabolic activity, one out of five rat oligodendrocytes OLN-93 were inhibited in their DNA synthesis. Their metabolism was found to be normal.

Table 1: BrdU test results.

| n=27, each $10^4$ cells | OLN-93 mean | OLN-93 SD | L-929 mean | L-929 SD |
|---|---|---|---|---|
| cell medium | 100 % | 2 % | 100 % | 2 % |
| neg. reference | 101 % | 7 % | 98 % | 5 % |
| platinum black | 81 % | 7 % | 98 % | 2 % |
| pos. reference | 0 % | 0 % | 0 % | 0 % |

Table 2: WST-1 test results.

| n=27, each $10^4$ cells | OLN-93 mean | OLN-93 SD | L-929 mean | L-929 SD |
|---|---|---|---|---|
| cell medium | 100 % | 3 % | 100 % | 4 % |
| neg. reference | 114 % | 4 % | 111 % | 10 % |
| platinum black | 99 % | 8 % | 100 % | 6 % |
| pos. reference | 0 % | 0 % | 1 % | 1 % |

## 4. DISCUSSION AND CONCLUSIONS

The described method for voltage-controlled electroplating of platinum black led to reproducible thin layers which uniformly coated the sputtered gold electrode contact. EDX analysis showed that the deposited platinum black material predominantly consists of platinum. However, the existence of traces of the toxic heavy metal lead could not be excluded. Other methods like X-ray fluorescence analysis may provide more detailed information on lead concentration.

A toxic effect, which might originate from potential lead in the platinum black was only found when DNA synthesis of rat oligodendrocytes was investigated by exposing the cells to an extract for 24 h. The same cell line was not affected in metabolic activity. Mouse fibroblasts were not affected at all.

Based on our findings, platinum black electrode coatings appear to be suitable for use in acute as well as in most chronic animal experiments. Implantation of platinum black material into the human body should be avoided, as long as other options can be considered.

### References

[1] Weiland JD, Anderson DJ, Humayun M. In Vitro Electrical Properties for Iridium Oxide Versus Titanium Nitride Stimulating Electrodes. *IEEE Trans. Biomed. Eng.*, vol. 49, 1574-1579, 2002.

[2] Ferris CD. Introduction to Bioelectrodes, Chapter 2.2. *Plenum Press London*, 13, 1974.

[3] Stieglitz T, Beutel H, Schuettler M, *et al.* Micromachined, Polyimide-based Devices for Flexible Neural Interfaces. *Biomedical Microdevices*, vol 2, 4, 283-294, 2000.