Biocompatibility Testing of Platinum Metallized Silicone Rubber

V. Vince¹, M-A. Thil¹, C. Dicara³, K. Kolev³, J. Delbeke¹, I. M. Colin², C. Veraart¹

¹Neural Rehabilitation Engineering and ²Histology Units, Université Catholique de Louvain (UCL), Medical School, Brussels, Belgium, ³Laboratoire de Physique de l’Etat Solide, Université de Mons-Hainaut (UMH), Mons, Belgium
valerie.vince@gren.ucl.ac.be

Abstract

Here we report on the biocompatibility testing of a silicone rubber metallized with platinum according to a new method. Both in vitro (Neutral Red Uptake) and in vivo (Subcutaneous Implantation) assays were used to demonstrate the cellular and systemic tolerance of the assessed material.

1. Introduction

Silicone polymers are widely used for biomedical applications, including cuff electrodes for nerve recording and/or stimulation. These electrodes were so far manufactured by hand, each contact being shaped and placed manually. This method results in a poor reproducibility for a given pattern and implies relatively limited designs. Progress recently made in thin conductive film deposition onto polymers (L.D.Laude–US Patent 5,599,592–EP 0693138–prior. 31/04/94) open new insights into cuff electrode manufacturing.

The SENS project (Smart Electrode for Nerve Sensing) aims at using this new technology to enlarge the field of existing recording electrodes with a system that can improve signal-to-noise ratio and selectivity.

The materials used for the SENS electrode, silicone rubber and platinum, are considered to be biocompatible and are widely used in implants. However, the three-dimensional shape of the platinum contacts of the SENS electrode is completely original since the metal is applied into grooves of the laser-processed silicone polymer as rough particles rather than as a smooth layer. Considering this new cuff-tissue interface, biocompatibility testing appears mandatory. Indeed, before any molecule, material, or device be used in close contact with biological systems, the host reaction might be tested. Any substance devoid of adverse effects is termed biocompatible.

2. Materials and methods

The protocol was approved by the Ethical Committee for Animal Experimentation (Medical School, Université Catholique de Louvain, Brussels, Belgium).

Materials

Rectangular samples (5mmx15mmx120µm) were assessed: Medical grade silicone rubber (MED4750, Statice Santé France with Nusil components) was irradiated with an excimer laser and subsequently metallized [2]. Untreated silicone MED4750 served as reference material. Samples were cleaned, steam sterilised (30 min, 120°C, 100 KPa), then cooled before use.

In vitro assay

A neutral red (NR) uptake assay, a method for assessment of biomaterial safety recommended by the International Organisation for Standardisation (ISO 10993-5 standard) has been performed. Confluent monolayers of BALB/c 3T3 fibroblasts were prepared in culture wells. Cells were incubated with a single sample for 24 hrs (37°C, 5%CO₂/95%O₂). Additional wells were used as negative (no sample), or positive (no sample, 6.4g/L phenol) controls. All conditions were repeated three times. After 24 hrs, the samples were extracted and the culture medium was replaced by NR (50 µg/ml [1], Sigma). After incubation (3 hrs, 37°C), the dye-containing medium was discarded and cells were fixed (0.5 % formaldehyde:1 % calcium chloride). They were examined with an inverted light microscope. NR was then extracted from the viable cells (1 % acetate:50 % ethanol). The optical density (OD, 540nm) of soluble neutral red was measured by spectrophotometry (Spectronic 1001, Bausch & Lomb).

In vivo assay

was performed in eight male Wistar rats after xylazine-ketamine anaesthesia. The dorsal skin was shaved, washed and disinfected before incision. Blunt dissection allowed to create subcutaneous pockets into which implants were inserted individually. After three months, the rats were sacrificed by transcardiac perfusion using 4% formaldehyde under deep pentobarbital anaesthesia. The implants with associated fibrous capsules were subsequently removed. The tissues were then embedded in paraffin, sliced at 5 µm, and stained with hematoxylin-eosin-safran (HES). The tissue response was evaluated according to the histologic appearance of the capsule surrounding the implants. (histology) Tissue characteristics were rated by histomorphometry [4].
3. Results

Microscopic observation (In vitro assay) of the cells and rating using a validate grading scale (Table 1) showed a mild toxicity for the metallized samples (index 1) compared to the positive control (index 3).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cell coloration</th>
<th>Decrease in cell density</th>
<th>Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>Present</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Positive Control</td>
<td>Absent</td>
<td>Marked</td>
<td>3</td>
</tr>
<tr>
<td>Silicone</td>
<td>Present</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Metallized</td>
<td>Present</td>
<td>Mild</td>
<td>1</td>
</tr>
</tbody>
</table>

OD measurements (In vitro assay) were averaged and presented as percentage of the negative control which received an arbitrary value of 100% (Table 2). A marked toxicity was observed for the positive control when compared to the negative one, e.g. a reduction in cell density greater than 90%, which validates the assay. For other test samples, there was no reduction in OD.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean OD</th>
<th>Standard Deviation</th>
<th>% Negative Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>0,709</td>
<td>0,084</td>
<td>100</td>
</tr>
<tr>
<td>Positive Control</td>
<td>0,035</td>
<td>0,051</td>
<td>4,9</td>
</tr>
<tr>
<td>Silicone</td>
<td>0,774</td>
<td>0,037</td>
<td>109,1</td>
</tr>
<tr>
<td>Metallized</td>
<td>1,020</td>
<td>0,163</td>
<td>143,8</td>
</tr>
</tbody>
</table>

Macroscopic evaluation All animals remained in good health during the 3-month recovery period: neither weight loss nor infection were observed. The wound healing was reached within a week post-surgery.

When the wounds were reopened, all implants were surrounded with a fibrous capsule (Figure 2), except the silicone implant of rat 8 which moved out of the subcutaneous pocket and joined the midline, under the sutures.

At the macroscopic level, blood was visible within the capsule of two metallized implants in rats 1 and 4 (not shown).

It was mostly impossible to separate implants from the surrounding fibrous capsule without causing damages to the adjacent tissues, as they adhered to the implanted material (Figure 3).

According to the NR assay criteria, a sample is considered cytotoxic if the cell survival index reaches a value of 2 or 3 (no incorporation or marked decrease in NR incorporation and/or marked decrease in cell density and/or marked modifications in cell morphology and/or marked detachment of the cells) during qualitative evaluation or if OD decreases by more than 25% of the control value during quantitative evaluation (Figure 1).

Based on these criteria, none of the test samples was considered as cytotoxic. The qualitative observations were correlated with the quantitative data.
Light microscopic observation of all tested samples showed a non specific tissular reaction. The implants were surrounded with a “two layer” capsule: the inner layer of fibrous tissue consisting of fibroblasts and collagen fibres and the outer layer less compact, more “reactive” contained mainly inflammatory cells, blood vessels, as well as fibroblasts (Figure 4).

Figure 4 Three months after the implantation, the capsule surrounding the implants was made of 1 an inner layer consisting of fibroblasts and collagen fibres and 2 an outer layer mainly containing inflammatory cells and blood vessels (silicone sample, rat 4).

Histomorphometric evaluation
A semiquantitative capsule classification was obtained by capsule thickness measurements based on the observed number of fibroblast layers (Table 3).

Table 3 Histological grading scale

<table>
<thead>
<tr>
<th>Thickness rating</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4 fibroblasts</td>
<td>4</td>
</tr>
<tr>
<td>5-9 fibroblasts</td>
<td>3</td>
</tr>
<tr>
<td>10-30 fibroblasts</td>
<td>2</td>
</tr>
<tr>
<td>&gt; 30 fibroblasts</td>
<td>1</td>
</tr>
<tr>
<td>Not applicable</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 5 Comparative rating of capsule thickness for silicone and metallized implants

Figure 5 shows the histomorphometric data for the capsule surrounding both types of implants after a three month implantation period. There was no statistical difference in capsule thickness between metallized and medical grade silicone implants (P > 0.05).

4. Conclusion

In conclusion, our data demonstrated that the new platinum silicone metallization process [2] does induced an aspecific inflammatory reaction similar to what is observed around more traditional electrodes [5]. No cytotoxic or other deleterious effects on adjacent cells and tissues has been observed.

This new platinum deposition process thus opens new perspectives for the repeatable construction of cuff electrodes carrying complex metal patterns.

Acknowledgment

This work was supported by the European Community (SENS project, n°QLG5-CT-2000-01372 in the frame of “Quality of life and management of living resources”), by the Région Wallonne (VISION project, n°114645) and by MFSR grant 3.4590.02.

References