A study of intra-cochlear electrodes and tissue interface by electrochemical impedance methods in vivo

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Abstract

This paper presents methods, results and analysis for measurements of the electrochemical impedance of platinum electrodes (~ 0.43 mm²) over a 6-month implantation in the cat cochlea. The study aimed to improve our understanding of the effects of tissue response on impedance behaviour. An increase in impedance in the post-operative period was evident with a rise of the distorted arc at high frequencies in the complex plane, correlating to anomalous charge transport at the electrode–tissue interface. The impedance at low frequencies generally showed a capacitive dispersion modelled as a constant phase element, indicating a blocking characteristic of the electrodes. The study suggests that a reduction and changes in composition of perilymph or extracellular fluid adjacent to the electrodes, as a consequence of tissue response, causes the elevated “contact impedance”. This affects the efficiency and quality of neural stimulating electrodes and neural recording electrodes. The finding of the crucial role of perilymph or extracellular fluid thin layer provides a new strategy for surface materials of neural electrodes, which is discussed in the paper. The interface characteristics must be considered during interpretation of studies undertaken in vitro or in acute experiments in vivo, where physiological fluid is abundant.

Keywords: Electrode–tissue interfaces; Intra-cochlear electrodes; Electrochemical impedance methods; Implantable device; Biomedical materials; Neural prostheses

1. Introduction

The multichannel cochlear implant utilises electric stimulation of the spiral ganglion cells in the human cochlea to provide acoustic information to profoundly hearing impaired adults and children. The cochlear implant converts frequency and intensity of sound into electric signals, which are sent to corresponding electrodes on the electrode array in the cochlea, stimulating nearby auditory nerve fibres [1] (Fig. 1a). While cochlear implants have been shown to provide speech perception benefits, results remain variable. In order to improve the efficiency of the cochlear implant and to develop new electrode arrays, there is the need for a reduction of electrode–tissue impedance and an increase in biocompatibility. Therefore, it is crucial to have an insight into the effect of tissue response on intra-cochlear electrode impedance. The study of the electrode–living tissue interface, including interface coupling and charge transfer characteristics through chronic experiments in vivo, can also provide important new knowledge for researchers of neural prostheses and biomedical materials.

Electrochemical impedance spectroscopy (EIS) is commonly used for characterisation of materials and electrode interfaces where ionic transfer is dominant [2]. EIS is one of the most sensitive and powerful techniques for the study of electrode–electrolyte interface. It has been used in studies of porous electrodes [3–5], conducting polymer-coated electrodes [6–8], thin film electrodes [9], DNA-modified electrodes [10] and implant materials [11,12]. In comparison to these extensive studies, there are relatively few reports of studies of the electrode–tissue interface using the EIS technique in vivo.

One such study of the platinum electrode–tissue interface in living mammalian tissue using perfused living rat heart [13]. When the electrode size was reduced
from 12.6 to 0.128 mm\(^2\), a small, depressed semi-circle appeared at high frequencies (HF) in the complex plane. This suggested thin film behaviour rather than a semi-infinite diffusion Warburg impedance. A second study of thin film iridium oxide electrodes (400–1600 \(\mu\)m\(^2\)) implanted in guinea pig cortex for up to 22 days also showed a depressed semi-circle at HF and a sloped straight line at low frequencies (LF) in the complex plane plots [14]. Two equivalent circuits were proposed to represent the electrode response in HF and LF. The influences of electric stimulation on impedance and charge storage capabilities were discussed. Despite these studies however, there is no general equivalent circuit model for an electrode–tissue interface, due to limited studies and variations of electrodes and biological environments. The effects of tissue response on implanted electrode impedance had not been well understood. Literature searches showed no AC impedance studies on chronically implanted intra-cochlear electrodes in vivo.

Charge transfer at a disordered materials interface generally exhibits spatially restricted diffusion behaviour, which can be described as finite diffusion control [15]. Recently, Bisquert et al. [3,16–18] have published a number of papers on the analysis of the AC impedance response to such systems. These papers extensively discuss ion transport in the hindrance thin layer and at the boundary (electrode double layer) using various transmission line circuit models. The present study adapts and modifies these models for analysing the AC impedance spectra of chronically implanted intra-cochlear electrodes in cats, where the electrolyte is non-homogeneous and undergoes changes. The analysis and discussion will focus on the understanding of the effects of tissue response on the electrode impedance and its implication to intra-cochlear electrode array materials.

2. Experimental

2.1. Animals and surgery

Two normal hearing adult cats were used in this study. At the same time, they were also used for an investigation of the effect of electric stimulation regimes. Each animal was bilaterally implanted, one side with a
stimulating electrode array and the opposite side with a control array. The lead wire was fixed on the skull and came out of the skin at the back of the neck. The details of the surgical procedure have been reported previously [19,20]. The care and use of animals reported in this study was approved and conducted under The Royal Victorian Eye and Ear Hospital’s Animal Research Ethics Committee Guidelines.

2.2. Animal intra-cochlear electrode array

Fig. 1b shows a schematic diagram of the Pt-banded electrode array used in the study. The intra-cochlear electrodes consisted of four 0.3 mm wide platinum rings on a Silastic carrier (Dow Corning medical grade elastomer MDX4-4210; Factor II, AZ, USA). The silicone rubber carrier tapered from a diameter of 0.43 mm at the tip ring to a diameter of 0.48 mm at the fourth ring, which results in the geometric area of each electrode as 0.41, 0.42, 0.44 and 0.45 mm², respectively. The cross-section of the array was approximately 0.15–0.18 mm². The electrode separation was 0.45 mm and the distance of each electrode centre was 0.75 mm. Teflon insulated platinum–iridium (90:10) wires connected each electrode to an insulated, stainless-steel lead wire. The lead wire provided external access to the electrodes for either impedance measurement or electric stimulation.

These electrodes were named E1–E4 from the tip, as shown in Fig. 1b. The four electrodes implanted in the cochlea are called intra-cochlear electrodes. The ball Pt electrode E0 (∼6 mm²) (Fig. 1b) is an extra-intra-cochlear electrode, and was implanted outside the bulla under muscles. Cat A was implanted with intra-cochlear electrodes only while Cat B was implanted with intra-cochlear electrodes and an extra-cochlear electrode. Fig. 1c gives a phase contrast X-ray image of an experimental cat implanted with the electrode array.

2.3. Electrochemical cell in vitro and in vivo

In vitro: 0.01 M phosphate buffered saline (PBS) was used, which contains 0.137 M NaCl, 0.003 M KCl. The pH is 7.4 at 25°C. Protein bovine serum albumin (BSA) (1% and 5% w/w) (Biosciences Pty Ltd) was added to the PBS for potential measurement. Physiological saline (saline) is 0.9% w/w NaCl (0.14 M). Deionised water (DIW) was used in preparing the solution and washing the electrode array.

In vivo: Fig. 1a schematically illustrates the human cochlea and human electrode array. The cochlea is a minute spiral compartment surrounded by bone. The bony labyrinth is divided by thin membranes into scala tympani (ST), scala media (SM) and scala vestibuli (SV). ST and SV contain perilymph and SM contains endolymph. ST is connected to the cerebrospinal fluid (CSF) of the subarachnoid space by the cochlear aqueduct. In the animal experiments, an implanted intra-cochlear electrode array was surgically inserted into the ST by perforating the round window (the entry to cochlea) and was in contact with perilymph. The maximum volume of perilymph around the electrode array depends on the anatomy of the ST. In a cat, the cross-sectional area of the ST falls from ∼5 mm² at the round window to ∼1 mm² at a depth of 6 mm, where the array tip is located. The space between the array and inner wall of ST is ∼0.4–0.7 mm at the tip of the electrode [21]. The chemical constitution of perilymph is similar to physiological saline [22], plus minor amino acids (∼0.6 mm) and proteins (∼0.2% w/w) [23,24].

All impedance spectra were measured in either a two-electrode cell or a three-electrode cell. Two-electrode cells use a pair of electrodes such as E1 and E3 or E1 and E0 as the working electrode (WE) and the counter electrode (CE), respectively. A saturated calomel electrode (SCE) or a Pt electrode in the array is used as a reference electrode (RE) in three-electrode cells. In experiments in vivo, SCE was inserted into a freshly cut muscle pocket at the back of the cat’s neck just before the cat was sacrificed. A large surface area Pt electrode was used as CE in experiments in vitro.

2.4. Electrochemical impedance measurements

An electrochemical interface (Solartron, Model SI 1287) and an Impedance Gain-Phase Analyser (FRA) (Solartron, Model 1260) were used in the study. All impedance spectra were measured at open-circuit potential using a 10 mV (rms) AC sinusoid signal. The frequency range chosen for most of the experiments was from 100 kHz to 0.1 Hz, although some were to 10 Hz. The current of the instrument was restricted to 20 μA by a cut-off mode. Usually, five frequencies per decade were chosen equidistant on a logarithmic scale. Zplot and Zview software were used in the measurement and curve fitting analysis (Scribner Associates, Inc.).

The impedance measurements were carried out on cats with or without anaesthetisation. EIS experiments had no adverse effect on cochlea function, suggested by a study of electrically evoked brainstem responses (EABRs) in guinea pigs, which will be reported in a separate paper.

2.5. Electrode preparation and measurement of open-circuit potentials

In vivo: The electrode arrays were ultrasonically cleaned in alcohol and DIW, and then sterilised prior to surgery. The open-circuit potential was measured when the SCE was inserted into a freshly cut muscle pocket at the back of the cats’ necks before the cats were
sacrificed. A few drops of saline were added to the pocket to make a good contact.

In vitro: The open circuit potential of the explanted electrodes were measured in 0.01 M PBS immediately after the termination of the cats. The electrode arrays were cleaned with a rinse, soaked in saline and then an ultrasonic bath in alcohol and DIW. Then the electrodes underwent cyclic voltammetry in 1M H2SO4 at a potential between −0.3 and 1.2 V vs. SCE for 10 cycles with 100 mV/s scan rate. All the experiments were carried out at room temperature and the cell was open to air. All the electrode potentials were measured vs. SCE.

3. Model

The equivalent circuit models of the intra-cochlear electrode–tissue interface are models of the electrode–electrolyte, with the difference that the electrolyte, living tissue, changes over time following the implantation. A general representation of the electrode–tissue interface is suggested in Fig. 2a. It consists of electrolyte impedance \( Z_S \) and electrode boundary impedance \( Z_B \). The Pt and perilymph/saline interface is approximately presented by a modified Randles equivalent circuit model, in which electrolyte resistance, \( R_S \), is in series connected to a constant phase element (CPE) \( Z_B \text{CPE} \) (Fig. 2b). The schematic diagram (Fig. 2c) is for the interface model A, in which \( R_S \) represents saline/perilymph. In model A, the faradaic charge transfer resistance is absent, as the Pt electrode shows blocking electrode behaviour when it is in contact with perilymph/saline at its open circuit potential. CPE represents dissipative double-layer capacitance and reflects the characteristics of a microscopic fractal at blocking liquid–solid interfaces.

Considering the fact that a sloped line similar to Warburg impedance or a distorted semi-circle appeared at Hf in the post-operative period, diffusive impedance \( Z_D \) replaces the electrolyte resistance \( R_S \) in model B (Fig. 2d). It is used to depict approximately the charge transport at the living biological electrolyte adjacent to Pt electrodes. The model C in Fig. 2e represents an adsorption occurrence on the electrodes. Thus, an adsorption resistance \( R_{AD} \) is added in parallel to the double-layer impedance \( Z_B \text{CPE} \). The diffusive impe-

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Fig. 2. Proposed equivalent circuit models for intra-cochlear electrode–living tissue interface. (a) General expression: \( Z_S \) electrolyte impedance; \( Z_B \) boundary impedance. (b) Model A: \( R_S \) electrolyte resistance; \( Z_B \text{CPE} \) interface impedance of CPE. (c) The schematic diagram is for the model A, in which \( R_S \) represents saline/perilymph; (d) Model B: \( Z_D \) diffusion impedance; \( Z_B \text{CPE} \) interface impedance of CPE. (e) Model C: \( Z_D \) diffusion impedance; \( Z_B \text{CPE} \) interface impedance of CPE; \( R_{AD} \) adsorption resistance at boundary. (f) An approximate representation of \( Z_D \) in the electrolyte phase (0 < \( x < L \)). \( x_1 \) is impedance per unit length (\( \Omega \text{ cm}^{-1} \)) and \( x_3 \) is the impedance length (\( \Omega \text{cm} \)) corresponding to the diffusion length \( L \) (cm). (g) A transmission line equivalent circuit of \( Z_D \). \( r_1 \) is the resistance per unit length (\( \Omega \text{cm}^{-1} \)), \( r_3 \) is the resistance length (\( \Omega \text{cm} \)) and \( q_3 \) is a CPE per unit length (\( F \text{ s}^{-1/2} \text{cm}^{-1} \)) within the distance \( L \). (g) The schematic diagram is for the models B and C, in which \( Z_D \) represents living biological material, but the layer is non-homogeneous and in poor contact with the electrodes.
dance $Z_D$ in Figs. 2d and e is generally expressed with the distributed equivalent circuit given in Fig. 2f. The elements $(r_1, r_3$ and $q_3)$ replace $x_1$ and $x_3$, and this results in the enhanced transmission line model (Fig. 2g) for $Z_D$.

A transmission line model usually represents the impedance response of a diffusion process. The distributed equivalent circuit has been used in characterising various systems where the charge transfer is controlled by a diffusion mechanism. Recently, Bisquert et al. [3,8,16,25–27] have carried out extensive analysis on these transmission line models and discussed the effects of boundary conditions ($Z_B$) and specific forms for $x_1$ and $x_3$ corresponding to material properties. In the present study, resistance and CPEs or their combinations replace $x_1$ and $x_3$ (Figs. 2f and g) in order to draw physio-chemical and biological properties, to give the best understanding to intra-cochlear electrode–tissue interface.

The diffusion impedance $Z_D$, represented by the general equivalent circuit model in Fig. 2f, is known as

$$Z_D = \lambda \cosh(L/o) = (x_1 x_3)^{1/2} \coth(L x_1 x_3)^{1/2},$$

$$\lambda = (x_3 x_1)^{1/2},$$

where $x_1$ is the impedance per unit length ($\Omega \text{cm}^{-1}$) and $x_3$ is the impedance length ($\Omega \text{cm}$) corresponding to the diffusion length $L$ ($\text{cm}$) [8,18].

The more specific equivalent circle model of $Z_D$ is illustrated in Fig. 2g, where $r_1$ replaces the $x_1$ and a parallel connection of $r_3$ and $q_3$ has replaced $x_3$. In the enhanced transmission line model, $r_1$ is the charge transport resistance in the diffusion length, and $r_3$ and $q_3$ denote the charge’s trapping or delay in the diffusion region. $r_1$ is the resistance per unit length ($\Omega \text{cm}^{-1}$) within the distance $L$. $r_3$ is the resistance length ($\Omega \text{cm}$) and $q_3$ is the coefficient of a CPE per unit length ($F \text{s}^{-1} \text{cm}^{-1}$). The impedance of $q_3$ named as $Z_{q3}$ is

$$Z_{q3} = \frac{1}{q_3 (j\omega)^{-\beta}},$$

where $j = \sqrt{-1}$, $0 < \beta < 1$, $\omega$ is the angular frequency (rad s$^{-1}$) = $2\pi f$, $f$ is frequency in Hz. When $\beta = 0.5$, the impedance is a Warburg impedance. The exponent sometimes deviates from the exact 0.5, signalling some type of anomalous diffusion process [16]. The above definitions give the expressions of the total resistance and diffusion CPE as follows:

$$R_1 = L r_1,$$

$$R_3 = r_3 / L,$$

$$Q_3 = L q_3,$$

where $R_1$ is a diffusive resistance. The combination of $R_1$ and $Q_3$ correlates to the trapping, motion or delay of local charges. The characteristic frequency of finite diffusion size $\omega_L$ corresponds to the transit time $t$, for a diffusing charge injected at distance $= 0$ to cover a distance $L$. It is defined as [8,18]

$$\omega_L = \frac{1}{(R_1 Q_3)^{1/\beta}} = \frac{1}{(r_1 q_3 L^3)^{1/\beta}},$$

(7)

$$f_L \approx \omega_L.$$  

(8)

The characteristic frequency $\omega_3$ of trapping or delay is given by

$$\omega_3 = \frac{1}{(r_3 q_3)^{1/\beta}} = \frac{1}{(R_3 Q_3)^{1/\beta}},$$

(9)

$$f_3 = \omega_3 / 2\pi.$$  

(10)

The two characteristic relaxation frequencies have the relationship based on Eqs. (7) and (9)

$$\frac{\omega_3}{\omega_L} = \left(\frac{R_1}{R_3}\right)^{1/\beta}.$$  

(11)

The impedance elements $x_1$ and $x_3$ therefore become

$$x_1 = r_1,$$

$$x_3 = \frac{r_3}{1 + r_3 q_3 (j\omega)^{-\beta}} = \frac{r_3}{1 + (j\omega / \omega_3)^{\beta}}.$$  

(12)

(13)

Combining Eqs. (1), (2), (4), (5), (10), (12) and (13), the diffusion impedance $Z_D$ becomes

$$Z_D = \left[\frac{R_1 R_3}{1 + (j\omega / \omega_3)^{\beta}}\right]^{1/2} \times \coth(j\omega_3 / \omega_L)^{\beta/2} \left[1 + (j\omega / \omega_3)^{\beta}\right]^{1/2}.$$  

(14)

Previous studies demonstrated that these parameters not only affect the features of impedance spectra but also give physico-chemical meaning to the system [3,8,18,25]. The effects of the characteristic frequencies on impedance spectra and the implications towards the cochlear–tissue interface will be discussed in this paper.

The diffusion impedance $Z_D$ model in Fig. 2g has previously been reported in other systems [8,25]. The literature shows that transmission line models are often used to depict thin layer restricted diffusion processes. However, the diffusion impedance $Z_D$ discussed here is correlated to the bulk origin, which is the living biological material adjacent to the electrodes. The living tissue as an electrolyte can dramatically change with respect to the distance to the electrodes over time. In this study, a pseudo-RE can be located as close as $\sim 0.7\text{ mm}$ to WE, which assumes that the diffusion length is within this distance. The series coupling between the diffusion impedance $Z_D$ and boundary, $Z_B$ shown in Figs. 2d and e, were the models that gave the best fitting results. The schematic diagram (Fig. 2h) is for the interface models B
and model C, in which \( Z_D \) represents living biological material, but the layer may be non-homogeneous and in poor contact with the electrodes.

In this study, three equivalent circuit models are used to depict the intra-cochlear electrode–living tissue interface under different circumstances. They are illustrated in Figs. 2b, d and e, and referred to as models A, B and C in this paper, respectively. According to the expression of \( Z_D \) in Eq. (14), the electrode impedances of these models A, B and C are expressed in Eqs. (16), (17) and (18), respectively. The curve fitting uses these models and equations, which is discussed in Section 4:

\[
Z_{\text{B-CPE}} = \frac{1}{Q_b} (j\omega)^{-x},
\]

\[
Z_E = R_S + Z_{\text{B-CPE}} = R_S + \frac{1}{Q_b} (j\omega)^{-x},
\]

\[
Z_E = Z_D + Z_{\text{B-CPE}} = \left[ \frac{R_1 R_3}{1 + (j\omega / \omega_3)^n} \right]^{1/2} \times \coth(\omega_3 / \omega_L)^{1/2} \left[ 1 + (j\omega / \omega_3)^n \right]^{1/2} + \frac{1}{Q_b} (j\omega)^{-x},
\]

\[
Z_E = Z_D + \frac{Z_{\text{B-CPE}} R_{\text{ad}}}{Z_{\text{B-CPE}} + R_{\text{ad}}} = \left[ \frac{R_1 R_3}{1 + (j\omega / \omega_3)^n} \right]^{1/2} \times \coth(\omega_3 / \omega_L)^{1/2} \left[ 1 + (j\omega / \omega_3)^n \right]^{1/2} + \frac{R_{\text{ad}}}{1 + R_{\text{ad}} Q_b (j\omega)^x}.
\]

These models all consist of electrolyte impedance and interface (boundary) impedance, and are arranged in series. A CPE, \( Z_{\text{B-CPE}} \), is used to depict a blocking interface, while a combination of \( Z_{\text{B-CPE}} \) and adsorptive resistance \( R_{\text{AD}} \) represents the adsorbing interface. The \( Z_{\text{B-CPE}} \) is given in Eq. (15), where \( j = \sqrt{-1} \), \( 0 < \alpha < 1 \), \( \omega \) is angular frequency (rad s\(^{-1}\)) = 2\( \pi \)f, \( f \) is frequency in Hz. In the case \( \alpha = 1 \), Eq. (15) recovers a perfect capacitor; when \( \alpha \) is close to 1, \( Q_b \) and \( \alpha \) are the coefficient of \( Z_{\text{B-CPE}} \) with dimension of F s\(^{x-1}\) and \( 0 < \alpha < 1 \). The CPE here represents a dispersive double-layer capacitance, which occurs in many real materials that are microscopically disordered [16].

4. Results and discussion

4.1. Influence of post-implantation time

The impedance spectra of a pair of intra-cochlear electrodes (E1 and E3) in cat A are displayed in Fig. 3. The complex plane plots clearly show changes in impedance with implantation time. A sloped line or distorted arc arises at Hf. This suggests that the electrolyte properties adjacent to the electrodes changed with time. In contrast, the impedance spectra at Lf remain unchanged, a steep straight line. This indicates a blocking electrode characteristic. It is worth noting that the impedance spectra of implanted electrodes immediately after surgery and the impedance spectra of explanted electrodes in PBS are quite similar, suggesting that the presence of perilymph around electrodes following the surgery significantly influences the impedance, in particular the impedance in the HF region.

The tissue conductivity is a sensitive function of the extracellular volume fraction [28,29], since the extracellular fluid is the conductive path. Although there is no reference for the conductivity of cat’s cochlea, the conductivity of bone (~1.4 \( \times \) 10\(^{-4}\) S/cm) is approximately 100 times lower than the conductivity of perilymph (~1.4 \( \times \) 10\(^{-2}\) S/cm, same as saline) [29].
maximum amount of available perilymph depends on the space between the array surface and the inner wall of the ST, which is approximately 0.4–1.2 mm [21]. However, the actual available perilymph immediately after surgery may be less than this owing to the leaking and slow replacement of perilymph [30,31]. Results in Fig. 3 show that such a thin layer of perilymph provided a similar conductance to bulk saline solution. The results also suggest that the increase in impedance is due to the changes in the electrolyte close to the electrode array. It is the fact that the constitution of the perilymph and the amount of perilymph could be largely influenced by the tissue response (inflammatory response).

A complex plane plot of the impedance at HF of an intra-cochlear electrode (E1) and an extra-cochlear electrode (E0) in cat B is given in Fig. 4. It shows similar tendency to those shown in Fig. 3. There were large increases during the first month following surgery and less change after the first month. This implies an unstable interface within a few weeks after implantation. These results demonstrate general characteristics of impedance. However, the data using two-electrode measurements can only provide a sum of each electrode’s interface and the electrolyte. Since electrode impedance also depends on electrode surface area and position of the electrode, it is very useful to study each electrode, enabling characterisation of the individual electrode interface and gain information on localised living biological environments and its effect on impedance.

4.2. Using pseudo-reference electrodes

The platinum band electrodes are fixed at a certain distance apart in intra-cochlear electrode arrays (Figs. 1b and c). The configuration of the electrode array provides the opportunity to use one neighbouring electrode as a pseudo-RE while no DC potential is applied. In the measurements, E2 and E4 implanted in Cat B were alternatively used as WE and CE, while the neighbouring electrode (E3) was an RE, as shown schematically in Fig. 5a. The results have been compared to the results obtained by using an SCE, which was carefully inserted into a freshly cut muscle pocket at the back of the cat’s neck just before the cat was sacrificed. The distance of the SCE is far away from WE and CE compared to pseudo-RE E3. The
impedance plots of each electrode measured with two types of RE agree with each other over the entire frequency range (Fig. 6). This has been seen in most experiments. It was also found that the sum of each electrode’s impedance agreed with the impedance measured with the two-electrode cell. This will be verified in the next section with curve fitting. The results denote that the equilibrium potential line of the remote SCE overlaps the centre potential line measured by the pseudo-RE E3 in the symmetric cell arrangement.

The distinct impedance spectra of E2 and E4 were detected by using E3 as pseudo-RE (Fig. 6). The experimental data show that E4 had high diffusion impedance, which is possibly associated with the presence of resistive bio-film on the surface. This may be related to the fact that E1, E2 and E3 had been electrically stimulated several hours every day for ~5 months while E4 had not been applied stimulation. Moreover E4 was located close to the round window, where fibrous tissue growth is severe [19].

SCE can only be used at the end of chronic experiments, just before the animal is sacrificed. Using pseudo-RE enabled the study of individual electrode–tissue interfaces in the post-operative period. Fig. 7 shows impedance plots of E1 and E0 using E2 as a pseudo-RE on days 1, 15 and 175. The arrangement of the electrodes in the measurement is illustrated in Fig. 5b. The impedance of E1 gives information on the electrode–tissue interface within a distance of approximately less than 0.75 mm. The results show that an increase in impedance is dominantly located near the intra-cochlear electrode.

4.3. Equivalent circuit models and curve fitting

The experimental results were initially fitted with several equivalent circuit models using software Zview 2.3e (Scribner Associates, Inc.). Since the electrode–living tissue interface varies for individuals with time,
the three models shown in Figs. 2(b), (d) and (e) give excellent fits for the experimental data. Model A is used to fit the impedance data measured in PBS/saline and in acute experiments (immediately after surgery). Models B and C are used for data in chronic experiments.

Representative experimental data and fitting data with corresponding models are displayed in Fig. 8. The figure shows a good fit over the entire experimental frequency range. The fitting models and mathematical expressions (Eqs. (16)–(18)) have been discussed previously. The two components of impedance, diffusion impedance \( Z_D \) and boundary impedance \( Z_B \) can be individually identified. Fig. 9 shows the complex plane plots and Bode plots of \( Z_D \) and \( Z_B \) for E2 and E4. The diagram shows that \( Z_D \) is frequency dependant at Hf and reaches a steady value at 10–100 Hz (Fig. 9c). \( Z_{D\text{-max}} \) is the \( Z_D \) approaching the maximum at below ~10 Hz. Fig. 9c reveals that \( Z_D \) and \( Z_B \) are dominant alternatively to total electrode–tissue impedance at Hf and \( f_L \), which is separated at ~100 Hz. The frequency where \( Z'' \) of \( Z_D \) and \( Z_B \) meet (Fig. 9f) is the characteristic frequency \( f'' \) shown in complex plane plots (Fig. 3). The impedance at frequencies greater than \( f'' \) is mainly contributed by electrolyte diffusion impedance \( Z_D \). It should be noted that \( f'' \) is different from the characteristic frequency of finite diffusion size \( f_L \) and the frequency of trapping and delay \( f_3 \). The latter parameters describe diffusion characteristics in the electrolyte phase and at interface.

Important experimental data and fit parameters, corresponding to Figs. 3 and 6, are summarised in Tables 1 and 2. The diffusion length “\( L \)” in the model is the distance between WE and RE. Although the distance of the two-electrode cell and three-electrode cell using a pseudo-RE is approximately 1.5 and 0.75 mm, respectively, non-homogeneity and complexity of the electrolyte around the electrode array makes it hard to identify the distance \( L \) and the cross-sectional area of the electrolyte. With the fitting, \( L \) is chosen as 1. Thus, \( R_1 \), \( R_3 \) and \( Q_{\text{B}} \) are used for a comparison of the impedance characteristics that were measured with the same arrangement. \( Z_{\text{HF}} \) is the fast response impedance of the bulk electrolyte at 100 kHz. \( Z_D \) is the frequency dependent electrolyte impedance and reaches the maximum value \( Z_{D\text{-max}} \), while frequency decreases (Eq. (14) and Figs. 9c and f). \( Z_{\text{HF}} \) and \( Z_{D\text{-max}} \) increase with implantation period shown in Table 1. \( R_1 \), \( R_3 \), \( Q_{\text{B}} \) and \( \beta \) correlate to the characteristic of charge transfer in the electrolyte phase on the electrodes. \( f_L \) and \( f_3 \) are the diffusive characteristic frequency and charge trapping or delay characteristic frequency, respectively. They determine the locus of \( Z_D \) in complex plane plot, as shown in Fig. 9b [25–27]. When \( f_L < f_3 \) and is in the measurable frequency window, the Warburg line with slope close to 1 is shown in Hf. The complex plane plot of E4 shows the slope at Hf region, while the slope of E2 is absent (Fig. 6). The \( f_L \) of E4 is 1.6 kHz but the \( f_L \) of E2 is far higher and out of the measurement frequency range (Table 2). These electrodes, having been stimulated for long periods, seem to show characteristics similar to E2. The high frequency arc is an uncompleted semi-circle. This may correlate to electrode surfaces where there is less tissue or cell adhesion, but body fluid is deficient.

Both experimental data and fit parameters denote that using a pseudo-reference enables identification of each electrode interface. The impedance of a pair of electrodes is the sum of impedance of each electrode–electrolyte that is separated symmetrically using such a cell configuration. The double-layer capacitance \( (C_{\text{dl}}, \gamma''/\omega) \) at 1 or 0.1 Hz of a pair of electrodes is approximately half of the average of each electrode (Table 2).

### 4.4. Impedance at high frequencies

The impedance at Hf is the impedance at frequencies of 1–100 kHz, where \( Z_D \) is dominant (Fig. 9c).
Impedance in Hf range is fairly important to intra-cochlear electrodes because electric stimuli of auditory nerves commonly employ biphasic current pulses with a typical duration of 25–100 μs. The impedance at Hf is meaningful for other type of neural stimulation as well [14]. The impedance at Hf increased significantly within a couple of weeks after surgery as shown and discussed previously. The $Z_{\text{Hf}}$ and $Z_{\text{f00}}$ of E1, E0 and their sum against time are plotted in Fig. 10. These were measured by the pseudo-RE E2 and represent the impedance at Hf region. The experimental data $Z_{\text{f00}}$ are close to the fitted data $Z_{\text{D-max}}$ as shown in Table 3 as well as in Tables 1 and 2. The result illustrates the trend of the changes in impedance at Hf and is thus apparent that most of the increased impedance is closely located in the intra-cochlear electrode within approximately 0.7 mm. The distribution of $Z_{\text{Hf}}$ and $Z_{\text{D-max}}$ vs. distance away from the intra-cochlear electrode E1 is plotted in Fig. 11, using E2, E3, E4 and SCE as the RE, respectively. The total distance of E1 to E0 and equal potential lines of SCE are approximately estimated (Figs. 1c and 5b). The complex plane plots of the impedance of E1 with these

---

### Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Z_{\text{Hf}}$</td>
<td>kΩ</td>
<td>0.98 2.4 3.9 5.6 0.98</td>
</tr>
<tr>
<td>$f^0$</td>
<td>kHz</td>
<td>- 4.0 6.3 6.3 -</td>
</tr>
<tr>
<td>$Z_{\text{f00}}$</td>
<td>kΩ</td>
<td>- 6.3 6.9 8.9 -</td>
</tr>
</tbody>
</table>

### Fit data

| $R_1$ | kΩ | — | 4.9 8.4 2.3 — |
| $R_3$ | kΩ | — | 5.3 4.7 8.6 — |
| $Q_0$ | μF s$^{-1}$ | — | 0.21 0.041 0.076 — |
| $\beta$ | 1 ≥ $\beta$ ≥ 0 | — | 0.60 0.68 0.55 — |
| $f_1$ | kHz | 95 124 >MHz |
| $f_3$ | kHz | 13 46 98 |
| $Z_{\text{D-max}}$ | kΩ | 0.98 5.4 7.2 9.3 1.0 |
| $Q_{\text{B}}$ | μF s$^{-1}$ | 0.28 0.33 0.20 0.29 0.31 |
| $\alpha$ | 1 ≥ $\alpha$ ≥ 0 | 0.80 0.73 0.85 0.75 0.77 |

**Fitting model**

| A | B | B | B | A |

**Note**: 179$: after being explanted and tested in PBS.
pseudo-REs shown in Fig. 12 further verify that the distorted Hf arc correlates to electrolyte properties close to the electrode and the coupling between electrolyte and electrode. The shape of these complex plane plots does not change much, but only shifts higher along the real impedance axis when moving the RE away from the electrode E1. It also should be noted that the electrode double-layer characteristic was not affected by using different REs, which is revealed by experimental data $Y_{00}$ and fit parameters $Q_B, a$ in Table 3.

The semi-circle-like complex plane plots at Hf were observed in polymer electrolyte [32] and on porous electrodes [33]. Of these electrode interfaces, spatially restricted linear diffusion [15,16] or anomalous diffusion within a thin film [15,27] is often described for the mass transport characteristics. In the present study, curve-fitting analysis suggests that the distorted arc at Hf correlates to diffusion impedance $Z_D$. Thus, the spatially restricted anomalous diffusion in the living electrolyte phase and at the interface region is attributed to the increased impedance at Hf. The implanted intracochlear electrodes demonstrate a very special electrode–electrolyte system. The body response to foreign implant and surgery trauma leads to many changes in the electrolyte within ST, including its chemical constitution and the amount of perilymph during the early post-implantation period. It was reported that macromolecules such as amino acids and proteins increased [23,31]. There is an increase in inflammatory cells [34].

### Table 2

Experimental data and fit parameters for the impedance spectra of a pair of electrodes and each electrode in Fig. 6

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Electrodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Z_{Hf}$</td>
<td>kΩ</td>
<td>E2–E4, E2$^a$, E2$^b$, E4$^a$, E4$^b$</td>
</tr>
<tr>
<td>$f^*$</td>
<td>kHz</td>
<td>— — — —</td>
</tr>
<tr>
<td>$Z_{r}$</td>
<td>kΩ</td>
<td>— — — —</td>
</tr>
<tr>
<td>$Y_{00}/(1\ Hz)$</td>
<td>μF</td>
<td>— — — —</td>
</tr>
<tr>
<td>$Y_{00}/(0.1\ Hz)$</td>
<td>μF</td>
<td>— — — —</td>
</tr>
</tbody>
</table>

### Table 3

Selected experimental data and fit parameters with model C for impedance of E1 with difference RE at day 175

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Reference electrode</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Z_{Hf}$</td>
<td>kΩ</td>
<td>E2, E3, E4, SCE</td>
</tr>
<tr>
<td>$f^*$</td>
<td>kHz</td>
<td>— — — —</td>
</tr>
<tr>
<td>$Z_{r}$</td>
<td>kΩ</td>
<td>— — — —</td>
</tr>
<tr>
<td>$Y_{00}/(1\ Hz)$</td>
<td>μF</td>
<td>— — — —</td>
</tr>
<tr>
<td>$Y_{00}/(0.1\ Hz)$</td>
<td>μF</td>
<td>— — — —</td>
</tr>
</tbody>
</table>

Note: The impedance spectra are displayed in Fig. 12.
Gradually, collagen fibres could increase and fibrous tissue encapsulation is likely to be present [19]. This causes considerable and complicated changes adjacent to the electrode array, not only in the properties of the electrolyte but also in the coupling of the electrolyte to electrodes.

A distinct difference in the intra-cochlear electrode interface at the first month after surgery and in the extended period is evident from the curve fitting. The fitting result in Table 1 shows that $Z_{D\text{-max}}$ increased with time. $R_1$ increased and then declined while $R_3$ was higher after 179 days than those measured within 3 weeks. $R_1$ is the diffusive resistance and $R_3$ is the resistance correlated to trapping, motion or delay of charges. Elevated impedance measured within the first month could be associated with the presence of bio-film, or packed inflammatory cells and fibrin on the electrode surface. The high impedance measured in the extended period is likely to be correlated to the poor contact between electrodes to fibrous tissue or to the inner wall of the ST. However, a deficiency of perilymph or extracellular fluid close to electrodes is a possible cause in both cases. The $Z_{D\text{-max}}$ was 1.0 kΩ for the explanted electrodes in PBS shown in the last column of Table 1, and the result was very close to the 0.98 kΩ for implanted electrodes in day 1. This shows the influence of aqueous electrolyte.

4.5. Impedance at low frequencies

The interface impedance (or boundary impedance) is dominant at Lf between several tens of Hz to 0.1 Hz (Fig. 9c). Here the electric signal needs a longer time to penetrate to the electrode boundary and sense its properties. The interface impedance associated with the charge dispersion correlates to either charge storage or chemical adsorption at the electrode double layer (interface boundary). Generally implanted intra-cochlear electrodes demonstrate a blocking boundary but an adsorbing boundary was observed sometimes.

The blocking interface characteristic was more often detected, being indicated by a capacitive response in the complex plane plot at Lf (Figs. 3 and 8). This blocking interface character is described as a CPE [16], which is an intrinsic electrode polarisation effect unrelated to faradaic currents crossing the interface [35]. It should be noted that the measurements were carried out at an open-circuit potential. The potentials of the stimulated electrodes should be close to the open-circuit potentials, because the output of the stimulator and electrodes are always shorted together between pulses to avoid build-up of potential difference across the electrodes (DC bias). The blocking electrode denotes that no redox
reactions occurred. The coefficients of CPE, $Q_B$ and $\alpha$, obtained by the curve fitting show very little change over time, suggesting that there is no obvious change in double-layer structure and therefore no changes in the real electrode surface area (Table 1). Results in Tables 2 and 3 show that the coefficient $Q_B$ agrees with the experimental data $Y''/\omega$ between 1 and 0.1 Hz, which is the electrode double-layer capacitance $C_{dl}$ at these frequencies. The study indicates that an increase in $C_{dl}$ leads to a reduction of impedance at the middle- to low-frequency range (Fig. 9c). This must benefit safe electric stimulation and neural signal recording.

The intra-cochlear electrodes, however, sometimes show an adsorbing boundary. Some impedance spectra show phase decline in the complex plane plot (E4 result in Fig. 8) and in the Bode plot at $L_f$ (Fig. 9d). The electrode E4 was implanted for 175 days but it had not been electrically stimulated. The equivalent circuit of the electrode includes a surface adsorption resistance (Fig. 2e), which fits the experimental data well (Fig. 8 and Table 2). $R_{SD}$ is an approximate circuit element as the impedance includes adsorption capacitance, but the influence of an adsorption capacitance is only significant in frequencies less than 0.1 Hz.

The decrease in phase angle of the complex plane plot at $L_f$ was observed in various electrodes, such as polymer-coated electrodes [7] and DNA-modified electrodes [10]. The presence of organic or inorganic substances may favour the energy dispersion at electrodes. Besides possible effects of adsorption, electrolyte properties can also affect the impedance at $L_f$. When a biological film is present on the electrode surface, it can affect the charge dispersion at $L_f$. The impedance is a mixed response of electrolyte and interface properties [16].

4.6. Effect of perilymph, extracellular fluid, protein and fibrous tissue on impedance and the implications on electrode array materials

The electrical properties of biological tissue are described by a suspension of cells or fibres in a saline solution. At frequencies less than 100 kHz, the current is restricted to the extracellular fluid and carried by the motion of free ions in response to the electric field [29]. The amount of extracellular fluid usually determines the conductance of a type of tissue or living organ. Bio-impedance was commonly used for monitoring extracellular fluid in tissue and organ [36].

The study discovered that the key impact of tissue response on impedance behaviour of intra-cochlear electrodes is attributed to a reduction of the perilymph adjacent to the electrodes. The initial tissue response is an acute inflammatory response. Increased vascular permeability leading to the accumulation of protein-rich extravascular fluid (exudate) is the hallmark of acute inflammation [37]. The slow replacement of perilymph and the increase in extravascular fluid lead to low impedance measured in a short period after surgery. Continued presence of the electrode array excites chronic inflammation, which is characterised by infiltration with mononuclear cells, such as macrophages and lymphocytes cells, and fibrous tissue growth [37]. These inflammatory cells, fibrin and fibroblast can accumulate on electrode surface in dense or loose form with the resolution of the exudate present. The development of tissue encapsulation further diminishes the fluid layer. It is clear that tissue response (inflammatory response) dramatically causes changes in the electrolyte close to the electrodes. Perilymph or extracellular fluid adjacent to the electrode array as “bulk electrolyte” diminishes either by the presence of packed biological cells or a miniature “gap”. This leads to poor electric contact of electrode to tissue or inner wall of ST. Elevated impedance possibly appears without the presence of fibrous tissue adjacent to the electrodes. Therefore, the primary reason accounting for marked increase in anomalous ionic diffusion impedance is the lack of adequate aqueous electrolyte at interface region. The growth of fibrous tissue, scar tissue or tissue encapsulation around the electrode array is the secondary factor, as it most likely leads to a further reduction of body fluid around electrodes.

There is comparatively less influence of tissue response on the charge dispersion at the electrode double layer. The interface boundary is ascribed to the electrode in contact with the physiological fluid, and the contact could be in the form of an extremely thin aqueous film, even in the form of moisture. It has been revealed previously that the double layer of intra-cochlear electrodes is characterised by a dispersive capacitance. However, the adsorption and rearrangement of macromolecules and biological cells could also affect impedance at $L_f$.

To verify the effect of the adsorption of protein, the adhesion of biological films and supporting electrolyte, electrodes explanted immediately and freshly cleaned were tested in PBS. The results are illustrated in Fig. 13 and Table 4. The explanted electrode’s surface evidently had residual adhesion of tissue, cells and proteins, as their open-circuit potentials were $68–160\text{mV}$ after several rinses with PBS, which was much lower than the $\sim 360\text{mV}$ of the bare, clean Pt intra-cochlear electrodes. The open-circuit potential of intra-cochlear electrodes ($n = 6$) in PBS was $369 \pm 4\text{mV} (\text{mean} \pm \text{SD})$, but reduced to $322 \pm 12$ and $187 \pm 1\text{mV}$ by adding 1% and 5% protein BSA, respectively. The open-circuit potentials were $–22 \pm 4\text{mV}$ when the electrode was in the cochlea just before the animal was sacrificed. All electrode potentials were with respect to an SCE reference electrode.
There are two distinctive characteristics between explanted electrodes and clean electrodes. The explanted electrodes show higher impedance at Hf and lower double-layer capacitance compared to the clean electrodes (Table 4). The complex plane plots of the explanted electrodes moves towards the right along the $Z''$-axis, indicating a small increase in resistance of 254 and 154 $\Omega$ at 100 kHz. However, the distorted arc and obvious increase in impedance as shown in the chronic experiments have not been observed (Fig. 13). The impedance discrepancy of explanted electrodes and clean electrodes demonstrates the influence of the presence of bio-film on the electrode surface when aqueous electrolyte is abundant.

The impedance at Hf is the main component of the impedance of intra-cochlear electrodes responding to electric stimulation. Obviously, it is significantly influenced by the presence of aqueous electrolyte, such as perilymph and extracellular fluid. The conductivity of perilymph and extracellular fluid is mainly ascribed to the ionic concentration that is similar to physiological saline. The results in vivo and in vitro manifest that the presence of such an aqueous layer adjacent to electrodes, prevailing to other factors of the presence of proteins, bio-films and tissue fibres on electrodes, determines impedance characteristics. The results suggest that the availability of perilymph or extracellular fluid on electrodes determines the impedance behaviour in acute experiments and in chronic experiments. Thus, a study carried out in a beaker or in acute experiments, where physiological fluid is abundant, does not reflect the occurrence in chronic studies in a living body.

Although the fact that tissue response causes increases in impedance is widely recognised in the literature, most efforts were made to suppress tissue response by drug delivery. It was reported that patients with steroid-eluting pacing electrodes showed stable pacing thresholds and sensed electrogram amplitudes throughout the 52-week follow-up period [38]. The stable electrode–tissue interface can be partially ascribed to the continuous presence of the aqueous fluid, but this is probably overlooked. The finding of the crucial role of perilymph or extracellular fluid in this study provides a new strategy for improving electrode–tissue interface, in order to achieve better electric coupling between cochlear implant and the auditory nerve fibres. The electrodes must provide high efficiency and low risk for charge transport at the electrode–tissue interface. Ideally, the electrodes should have porous surface and also include a layer of novel materials that provides the electric coupling and good biocompatibility to inner wall of ST of cochlea (or to neural tissue). There is also the need to promote water retention around the electrode array. The hydrophilic properties may be improved by grafting an ultrathin hydrogel film onto the silicone carrier surface.

5. Conclusion

Electrochemical impedance is a sensitive and powerful tool in the investigation of the charge transport characteristics at the intra-cochlear electrode and tissue interface in vivo. An increase in impedance is evident by the rise of the deformed arc in the complex plane plot at

![Fig. 13. Complex plane plots of impedance in 0.01 m PBS. The intra-cochlear electrodes were tested after having been explanted immediately for E1 (•) and E3 (▲) and after cleaning for E1 (○) and E3 (△). (a) Large-scale view (100 kHz–0.1 Hz) and (b) small-scale view (100 kHz–398 Hz).](image)

<table>
<thead>
<tr>
<th>Electrodes</th>
<th>$E_c$ (mV)</th>
<th>$Z_{Hf}$ ($\Omega$)</th>
<th>$Y''/\omega$ (1 Hz) ($\mu$F)</th>
<th>$Y''/\omega$ (0.16 Hz) ($\mu$F)</th>
<th>$R_s$ ($\Omega$)</th>
<th>$Q_n$ (\mu F s$^{-1}$)</th>
<th>$\varepsilon (1 \geq n \geq 0)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) E2</td>
<td>68</td>
<td>595</td>
<td>0.48</td>
<td>0.56</td>
<td>600</td>
<td>0.63</td>
<td>0.85</td>
</tr>
<tr>
<td>E4</td>
<td>160</td>
<td>520</td>
<td>0.50</td>
<td>0.60</td>
<td>525</td>
<td>0.67</td>
<td>0.84</td>
</tr>
<tr>
<td>(2) E2</td>
<td>333</td>
<td>341</td>
<td>0.59</td>
<td>0.84</td>
<td>308</td>
<td>0.92</td>
<td>0.78</td>
</tr>
<tr>
<td>E4</td>
<td>363</td>
<td>366</td>
<td>0.63</td>
<td>0.77</td>
<td>345</td>
<td>0.85</td>
<td>0.82</td>
</tr>
</tbody>
</table>
Hf, which correlates to anomalous ion diffusion in the electrolyte phase close to electrodes and on electrodes. This is mainly due to a deficiency of perilymph or extracellular fluid adjacent to electrodes as a consequence of tissue (inflammatory) response although the electrode surface is still moist. The electrolyte properties have significantly changed but the electrode double layer (boundary) retains the solid–aqueous feature. The growth of fibrous tissue or scar tissue around the electrode array is the secondary factor, as it likely leads further reduction of the fluid at the interface region. The presence of a thin layer of aqueous electrolyte, in fact perilymph or extracellular fluid, on intra-cochlear electrodes substantially influences the impedance behaviour. This key fact also determines the distinct impedance characteristics in acute experiments and in chronic experiments in vivo. We should consider the interface characteristics during interpretation of studies undertaken in a beaker or in acute experiment, where physiological fluid is abundant.

The double layer of the intra-cochlear electrode usually demonstrates a frequency dispersion showing a blocking interface characteristic. An adsorbing interface is found in some experiments owing to the presence of various proteins, bio-films or fibrils on the electrode surface. The electrode boundary condition only affects the impedance at Lf. Tissue response lowers the efficiency of neural electric stimulation, but meanwhile it reduces the risk of the occurrence of water electrolysis or other faradaic reactions at the electrodes.

It is highly desirable to improve the intra-cochlear electrodes by surface modifications. The study suggests that the electrodes should have porous surface and also include a layer of novel materials, which provides the electric coupling to neural tissue but is not affected by tissue response. In addition, there is the need to promote hydrophilic properties of the silicone rubber carrier, such as grafting an ultrathin hydrogel film onto the surface.

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References