

An Electrical Model for Off-Plane Nano Needle Array Electrodes in Intracellular Signal Measurement in Biological Environments

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Abstract

Electrical signals emanating from biological cells can convey clinical information on the functionality thereof. However, measurement of such small signals caused primarily by ionic activity inside the cell, known as action potential, poses a great challenge to biomedical scientists. The electrical signals of the biological cells result from exchange of ions through the cell membrane. The characteristics of action potentials may reveal a great deal of information about the causes and symptoms of abnormal cell behaviour. Hence, it is imperative to capture high quality action potentials through the use of nano-sensors from within the cell. Recently, developments in silicon nanowires (SiNW) fabrication techniques have demonstrated a great potential for them to be used as nano-electrodes. Large-scale assembly and integration of addressable complementary silicon nanowires arrays have been demonstrated for multiplexed biosensor arrays. The fabrication process resulted in a high-yield, high performance devices arrays for chemical and biological detection.

In this paper, we seek to model the electrical interface that is responsible for recording the biological signals. We present electrical equivalent circuits that model the boundary between the biological cell and the nanowire electrode. Impedance measurement curves of nanowires for various sizes of length and diameter have also been presented and discussed. The impedance graphs show a hyperbolic dependence of resistance on length and diameter of nanowires. This non-linear behaviour may be mitigated in software algorithms when interpreting the measured cell signals.

Keywords: Bio-electronics; Nano-electronics; Nanoneedles; Electrophysiology; Nanosensors; Biosensors; Electrical modelling

Introduction

Presently, electronics is being widely used in extremely sophisticated applications, such as neural signal recording and Brain-Machine Interfaces (BMI) which enable the manipulation of prosthetic limbs and other neuroscience applications [1,2]. The central goal of these and similar devices is to understand the physiological or pathological functions of neural circuits [3]. However, the capabilities of current electrophysiological technologies, such as patch electrodes, microelectrode arrays (MEAs) etc., need to be improved to allow simultaneous intracellular recordings. The intracellular recording systems provide accurate readout of the voltages generated by individual cells as compared to extracellular recording devices [4]. Intracellular recordings of neuronal Action Potential (AP) and Synaptic Potential (SP) may reveal a great deal of information as to the role of individual cells in certain functions or dysfunctions [3,5].

Recently, silicon nanowires (SiNW) have emerged as bio-electronic nano-sensors because of precise control in their synthesis and formation of arrays [6,7]. A typical SiNW generally has a diameter between 3 and 500 nm and length from several hundred nanometres to millimetres. Nanowires have been fabricated in various forms and shapes including homogeneous NWs, axial modulated, radial/coaxial modulated, branched or tree-like, and kinked structures [6,8]. Due to their high thin structure, SiNWs can penetrate deeply into single living cells for membrane potential readouts. This capability may further be utilized for internal drug delivery, electrophysiology and internal endoscopy [9]. Kubota et al. have demonstrated reliable acquisition of neural signals of a mouse's brain using nanoscale-tipped SiNWs in their experiments [10]. Lee et al. have also demonstrated interfacing of 60-nm diameter vertical SiNWs with living cells, thus measuring a steady-state average peak voltage of 10 mV [11].

Measurements of action or synaptic potentials with vertically-aligned SiNWs (nanoneedles) have certain advantages over other conventional techniques. The main advantage is a high signal-to-noise ratio and better temporal resolution as the nanowire is in direct contact with the cell membrane in contrast to other extracellular measurement methods where probes do not penetrate into the cells, and thus, do not record action potentials with the required details [12]. Furthermore, SiNW-based probes can detect sub-threshold potentials across the cell membrane, which is not possible by other conventional methods. Another advantage is that cell death or degradation does not occur when a nanoprobe is removed from the cell as compared to other methods, such as glass micropipette [13]. Aside from these advantages, the SiNW-based method still needs a precise equivalent circuit model, hi-fidelity signal wires, precise filtering, and highly-accurate data acquisition setups to capture and interpret cell membrane potentials in real time.

The paper is organised as follows. Section II presents the overall cell signal measurement system using nanowires. Section III proposes electrical equivalent models of various parts of the system, including the cell membrane and nanowire. Circuit simulations and results are given in Section IV. Section V and VI present discussion on results and conclusions of this research, respectively.

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Intracellular Signal Measurement Using Nanoneedles

In experiments carried out by our collaborators [14,15], SiNWs were fabricated on a silicon wafer using CMOS fabrication process in foundry. The nanowires were then coated with 150 nm Gold (Au) and a 5 nm Titanium (Ti) layer for adhesive support by magnetron sputtering, as shown in Figure 1a and 1b. A cross-sectional view of SiNWs using a scanning electron microscope (SEM) is shown in Figure 1c. A passivation layer is then deposited all over the structure to prevent contamination from external environment. Next, cultured tissue samples were spread over the whole structure of nanowires. An SEM view in Figure 1d shows the extent of penetration of nanowires into tissue cells. The manufactured wafers containing SiNWs were placed under a Teflon frame with a reference Silver/Silver Chloride (Ag/AgCl) electrode which was manufactured evaluated and calibrated (Figure 2a). In one of the experiments, eight such wafers were prepared and tested for cell membrane voltages in a cell culture incubator at 37°C with 5% CO₂ atmosphere. Results are shown in Figure 2b.

The experiments showed that SiNWs have successfully measured the membrane potential which can be amplified and then interpreted using specialised signal processing methods. However, precise electrical equivalent models for cell membrane, nanowire and complete

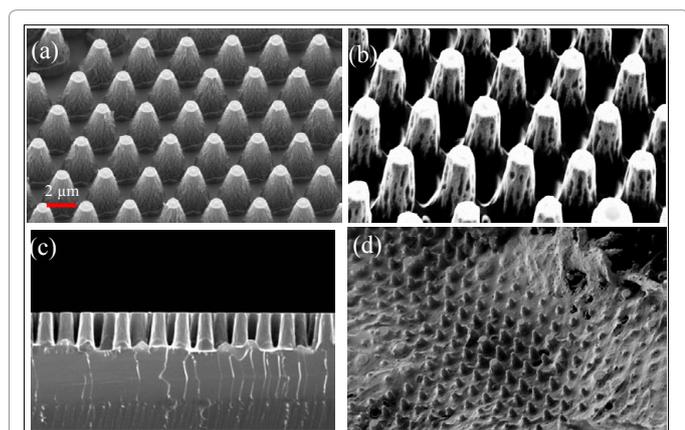


Figure 1: (a) Array of Silicon nanowires fabricated on silicon wafer (b) sputtering 150 nm Au and 5 nm Ti (c) a cross-sectional view of fabricated SiNWs (d) penetration of SiNWs into cultured living cells [15].

measurement process are needed to fully understand the meaning of measured action and synaptic potentials.

Equivalent Electrical Model

a. Cell membrane

A typical neuronal cell contains a cell membrane and ion channels Figure 3a. The channels open and close, depending on external stimuli. When a channel is opened, ions flow through the membrane and create a potential difference across a cell membrane. However, the opening of a channel is selective and dependant on the type of ions, e.g. Sodium (Na⁺), Potassium (K⁺). The channel selectivity may be regarded as equivalent to a high resistance while the membrane layers form a capacitor for charge storage. The selective permeability of cell membrane to various ions is the source of energy and may be modelled as a battery in electrical terms [16]. So, the equivalent circuit model of a cell membrane reduces to an RC circuit with a battery, as shown in Figure 3b.

In Figure 3b, Q₀ represents the equilibrium membrane potential. The built-in potential across membrane due to certain ions can be found by solving the circuit for membrane potential using Kirchoff's Voltage Law (KVL). Assuming only K⁺ channels in the membrane, we can write the following equation at equilibrium

$$\frac{c_o}{c_i} = e^{\frac{zFV_K}{RT}} \quad (1)$$

Where, c_o and c_i are molar concentrations outside and inside the membrane, R is the molar gas constant (8.314 J/K.mol), V_K is the equilibrium membrane potential due to Potassium ions, zF is the number of charges per molar ion, and T is the temperature. Solving for V_K, we get the following equation

$$V_K = \frac{RT}{zF} \ln\left(\frac{c_o}{c_i}\right) = 2.3026 \frac{RT}{zF} \log_{10}\left(\frac{c_o}{c_i}\right) \quad (2)$$

Eq. 2 is known as *Nernst Equation* and determines the equilibrium membrane potential contribution known as the *Nernst Potential*. Typical *Nernst* potentials for Na⁺ and K⁺ ions at equilibrium are +67 mV and -98 mV respectively.

b. Nanowire

A nanowire is a microsize electrical conductor able to sense an extremely low signal. A nanowire can be modelled as a parallel resistor-

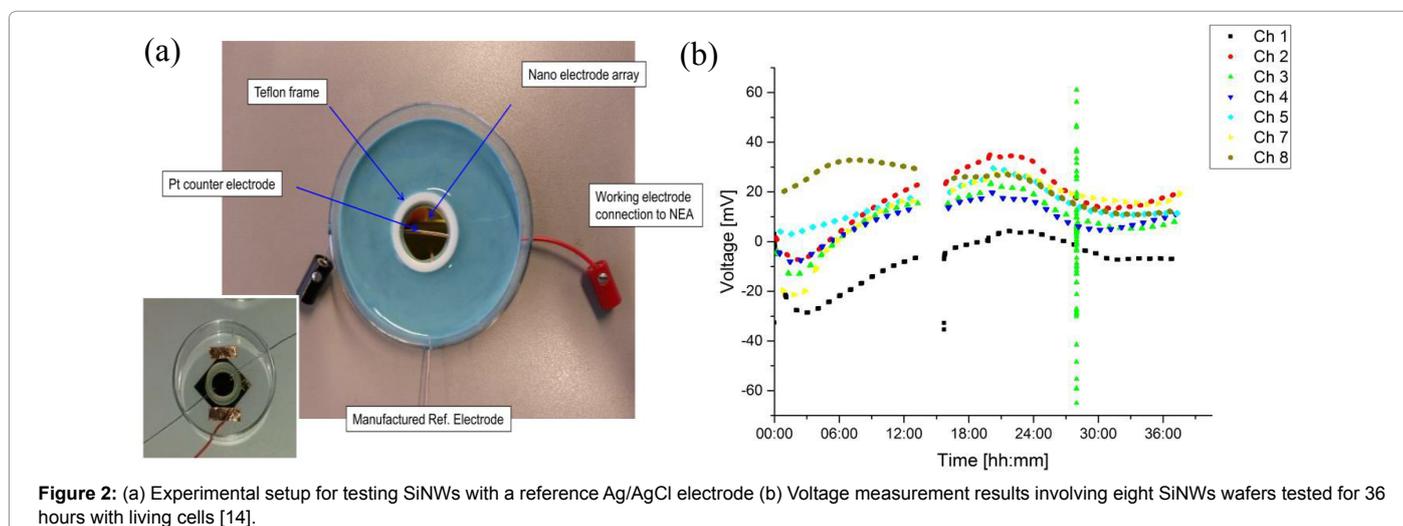


Figure 2: (a) Experimental setup for testing SiNWs with a reference Ag/AgCl electrode (b) Voltage measurement results involving eight SiNWs wafers tested for 36 hours with living cells [14].

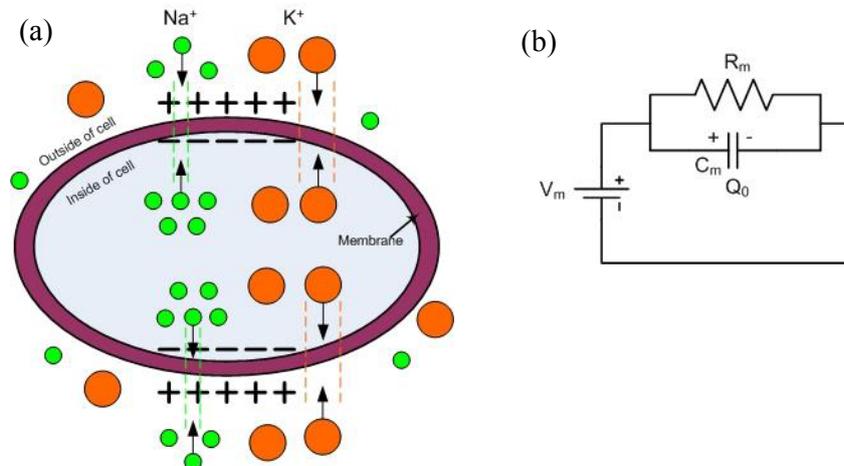


Figure 3: (a) An electrochemical representation of a cell membrane showing the ions and their selective channels (b) an equivalent circuit model of the cell membrane.

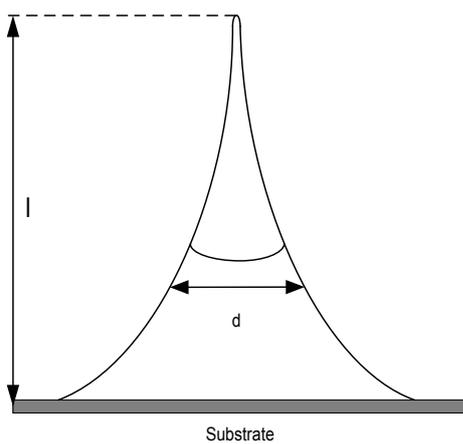


Figure 4: Approximation of a nanowire having length l (μm) and diameter d (μm).

capacitor (RC) circuit. Suppose, we have a nanowire with length (height) l and diameter d , both in μm . The structure of a nanowire is shown in Figure 4.

The diameter d is not uniform throughout the length of the nanowire, but it is approximately a hyperbolic function of wire length i.e. the diameter decreases with length and vice versa. Hence, we can write:

$$d(l) \propto \frac{1}{l^n} \quad (3)$$

where, n determines the non-linearity factor in the length. For simplicity in calculations, we can assume $n=2$. Eqn. (3) can be modified as:

$$d(l) = \frac{D_0^3}{l^2} \quad (4)$$

where, D_0 represents the diameter at $l=0+$. It is important to mention here that the wire length cannot be zero in this model for the base diameter D_0 , however, it is in the order of nm.

Let us consider the case of a nanoneedle made of pure Silicon without impurities added. Each material has a specific resistivity denoted by ρ and is measured in $\Omega\text{-m}$. The value of ρ for pure Silicon is

0.03 to 0.06 $\Omega\text{-m}$. The overall resistance of the nanowire can be found by the following standard equation:

$$R_{nw} = \rho \frac{L}{A} \quad (5)$$

Where, L is the total length and A is the cross-sectional area of the nanowire. The variable length of Silicon nanowire is l and the area at l is equal to the area of a circle i.e. $\pi \frac{d^2}{4}$, where d is the diameter. Inserting the values of L and A in eqn. (5), we obtain the following expression:

$$R_{nw} = \int_0^L f(l) dl \quad (6)$$

$$f(l) = \frac{4\rho_{Si}l^5}{\pi D_0^6} \quad (7)$$

c. Complete model

The proposed complete circuit model involves the cell membrane, its ion channels, silicon nanowire, signal wires and attached amplifier. The cell is represented by its equivalent model as shown in Figure 3b. A schematic of the overall model is shown in Figure 5.

The typical values of R_{cell} and C_{cell} are 0.1-1 G Ω and 0.1-5 pF, respectively [3]. The resulting range of time constant ($\tau=R_{cell} \times C_{cell}$) is 10 μs -5ms. The nanowire is normally reading the membrane potential through a cleft separator, which gives rise to a resistance called the seal resistance (R_{seal}). Typical values of seal resistance are 1-100 M Ω . The signal generated by the cell is attenuated by the seal resistance before being captured by the nanowire. The resistance of a nanowire is dependent on its shape determined by diameter and length, and is calculated using eqn. (6) as given above. The stray capacitance of the nanowire (C_{nw}) can be deemed negligible as there is no parallel plate effect with a single nanowire. However, there are coupling capacitances involved among all adjacent nanowires, with air as dielectric medium. The coupling capacitance results in interference and interleaving of captured cell signals. This paper does not discuss the effects of coupling capacitances. The signal captured by the nanowire is further distorted by the substrate capacitance (C_{sub}).

Circuit Simulation Results

The equivalent circuit is simulated using MATLAB Simulink tools.

As MATLAB does not provide specific toolboxes for bio-electronics, we used and adapted available toolboxes. Various elements of the circuit model have been chosen from available Simulink libraries such as, *SimScape/Electrical*, and *SimPowerSystems*. The block diagram of the simulated circuit model is shown in Figure 6 below.

The biological cell generates a pulse train, known as action potential, which typically consists of sharp charging and discharging pulses separated by a few milliseconds in time. The amplitude of the cell signals is in the order of millivolts (approx. -50 to +40 mV) if measured intracellularly, and in the order of microvolts if measured extracellularly [17]. The signal is attenuated by the impedance of the nanowire which is calculated using eqn. (6) given above. Since the impedance varies

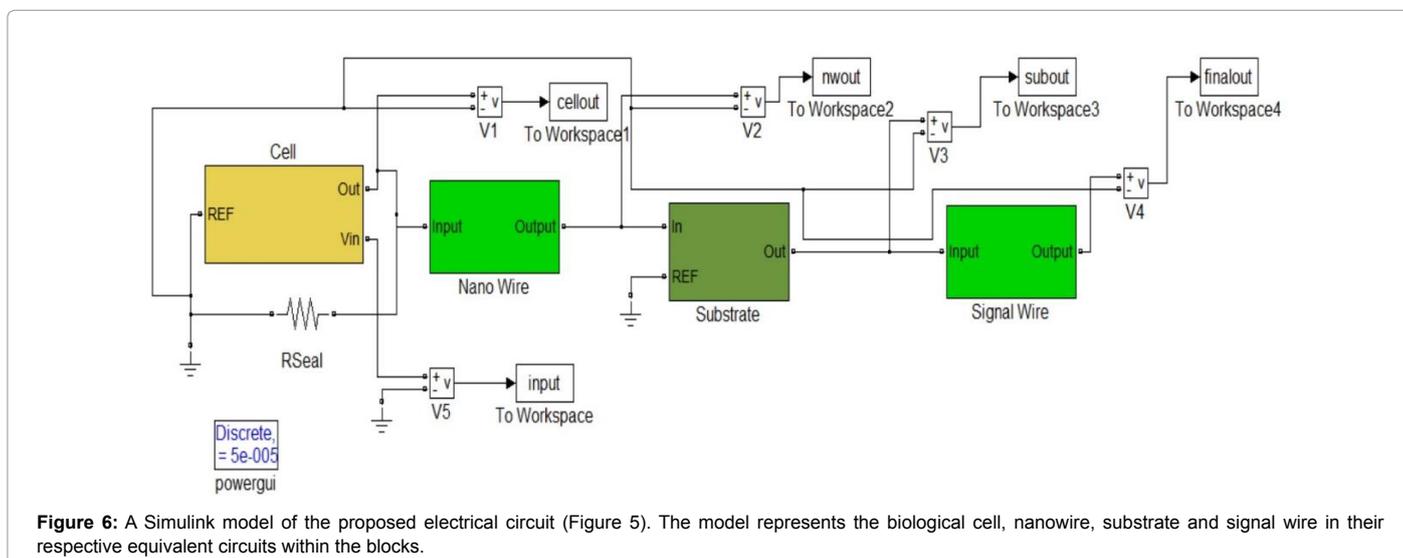
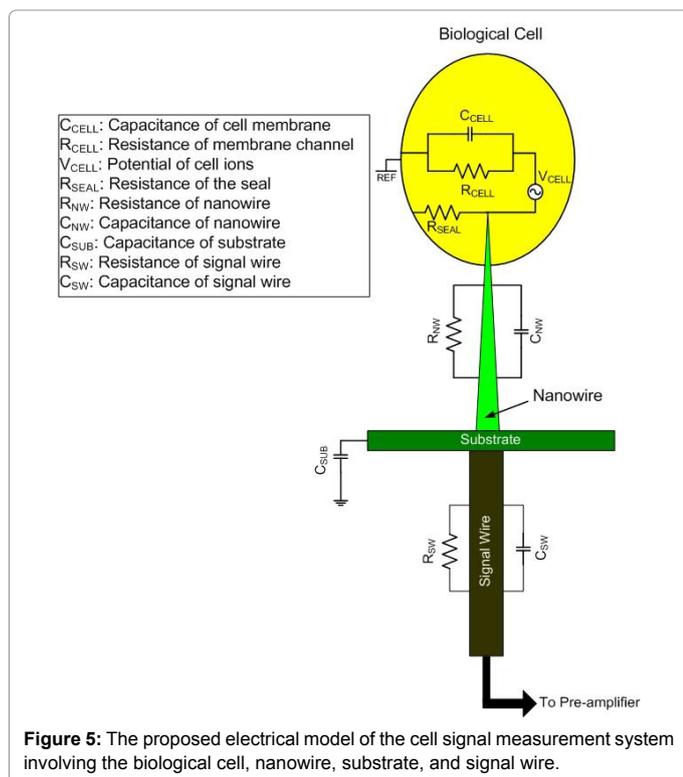
with both length and base diameter, impedance graphs are plotted against both quantities. Impedance graphs are also plotted for an imaginary nanowire having uniform diameter throughout its length. All the graphs are shown in Figure 7.

Discussion

The Silicon nanowire is best modelled by a resistance with a negligible stray capacitance. Figure 7a shows the variation in resistance with length of a hypothetical nanowire assuming a uniform cylindrical-shaped structure having 2 μm diameter. As the structure is uniform, the impedance varies linearly with length. In comparison, the impedance of a non-uniform conical-shaped nanowire varies super-linearly with length. As the length increases, the diameter decreases, hence the impedance of the nanowire increases also as a result of diameter shrinking (Figure 7b). This figure indicates that there is no significant change in impedance until around 5 μm length with base diameter fixed at 2 μm . However, the impedance increases sharply beyond that, and likely becomes obstructive to small signals. It is also evident from Figures 7a and 7b that the impedance of a conical-shaped nanowire is much larger (~ 1000 times) than a cylindrical-shaped rod with the same base diameter.

The variation in resistance of a cylindrical rod with diameter for a fixed length is shown in Figure 7c. It is clear that by increasing the diameter, the resistance decreases as $1/L$ function. However, there is no significant effect on resistance beyond the diameter size of 5 μm . Figure 7d shows the resistance vs. base diameter graph for a fixed length of a non-uniform cylindrical nanowire. The graph shows a steep hyperbolic fall in resistance with increasing diameter. The resistance is almost constant for diameters 3 μm and above, provided the length of nanowire remains fixed at 5 μm . Comparing Figure 7c and 7d also reveals that the resistance of conical-shaped nanowire is much larger (~ 1000 times) than that of a cylindrical nanowire for diameters below 2 μm .

Impedance curves in Figure 7 explain the variation of resistance with length and diameter. This variation in resistance is expected to produce non-uniform attenuation in the captured signal. It is expected that not all nanowires on a single wafer have the same length and base diameter due to variations in the manufacturing process. This means that the scale of the data measured by different nanowires will be different. For



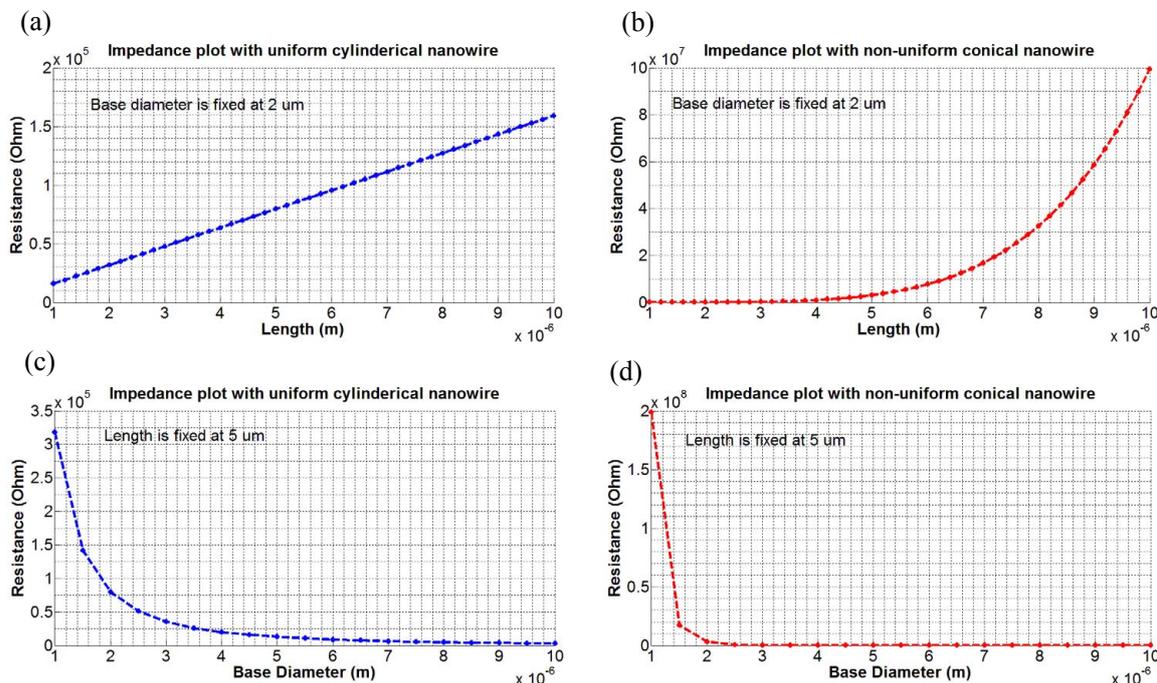


Figure 7: (a) Resistance vs. length for a uniform nanowire with 2 μm base diameter (b) Resistance vs. length of a non-uniform nanowire with 2 μm base diameter (c) Resistance vs. Base diameter of a uniform nanowire with 5 μm length (d) Resistance vs. Base diameter of a non-uniform nanowire with 5 μm length.

example, a nanowire is measuring 30 mV on a certain cell while its adjacent nanowire might be measuring 20 mV due to slightly shorter length or diameter. In a biological environment, such as neuronal cells, a small change in measured cell potential may be interpreted as a significant change in cell behaviour. Hence, modelling these effects allows for proper accounting of variability in the manufacturing processes. Suitable techniques can then design to mitigate the effects of non-uniform attenuation in cell signal measurement. This may be performed with the aid of software at the node where cell signals are processed and interpreted.

Conclusion

In this paper, we have presented the electrical equivalent circuit of silicon nanowire electrodes intended for biological cell signal detection. Silicon nanowires have been fabricated with various lengths and diameters, and initial experiments have confirmed their potential in electrophysiology precision measurements. The paper also presents electrical circuit models for a typical biological cell interfaced to a silicon nanowire. Impedance calculations of silicon nanowires have been presented as well against various related parameters such as length and diameter for different shapes. The impedance graphs show a hyperbolic dependence of resistance on length and diameter of nanowires. This non-linear behaviour may be mitigated with software while interpreting measured cell signals.

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