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Experimental evaluation of electrical conductivity of microtubules

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Abstract Microtubules (MTs) are self-assembled proteinaceous filaments with nanometer scale diameters and micrometer scale lengths. Through conductivity measurements in microchannels we shed some light on electrical properties of microtubules. Measuring electrical resistance we were able to detect the dynamic disassembly of MTs and determine an upper limit for the electrical conductivity of MT. The measurements yielded the value of 90 Ω^{-1} m^{-1} as the upper limit for the conductivity of MTs, which is in the order of conductivity observed in inorganic intrinsic semiconductors.

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Introduction

Microtubules (MTs) are proteinaceous tubes with 24 nm external diameter and micrometer lengths formed by a self-assembling process. Microtubules are one of the major components of the cytoskeleton of eukaryotic cells and serve a number of critical cellular purposes. MTs are biopolymers assembled from two, related protein monomers; α and β tubulins [1]. In the presence of the small molecule of guanosine 5′-triphosphate (GTP), these tubulin monomers form a heterodimer, which self-assembles into the microtubule structure. Due to the geometry of self-assembly and differences in addition rates, a MT is chemically polarized containing (–) and (+) ends. The (–) end contains an exposed α tubulin and undergoes slower heterodimer addition rates than the (+) end, which consists of an exposed β tubulin. Microtubules generated from pure tubulins exist in a dynamic state with net addition of monomers to the (+) end and net removal of monomers from the (–) end [2]. This ''treadmilling'' effect can be controlled via interaction of the MT with various chemical agents (e.g. taxol) resulting in stable MTs of fixed length. In the absence of these agents and with a shortage of monomers, MTs will disassemble. For tubulin concentrations above a critical value, C_c, tubulin dimers polymerize into MTs while below C_c, MTs depolymerize [1]. Near the C_c, MTs exhibit dynamic quasi-equilibrium during which an MT undergoes apparently random successive periods of assembly and disassembly. This dynamic process of assembly and disassembly is an intrinsic property of MTs. Several proteins, called microtubule-associated proteins (MAPs) have MT-binding activity. The assembly of MAPs prevent MTs from depolymerizing and

help organize them into parallel arrays, spindles, bundles, and other hierarchical structures [3].

MTs perform many important functions in the cell ranging from transport to cell division. For instance, MTs serve as tracks in the intracellular transport of vesicles, proteins and organelles powered by motor proteins (e.g. kinesin) [1]. In addition to the well-known chemical and cargo transport capabilities of MTs, several recent publications have conjectured signal propagation or electrical conduction along MTs. A simple model based on dipole interaction (each tubulin dimer is believed to have an electric dipole) was proposed to explain a mechanism of signal propagation [4]. Some indirect evidence for electrical conduction along MTs via electron or proton transfer is also reported [5]. Preliminary results of calculations of simple quantum mechanical models suggested that MTs could conduct electrical currents with electrical conductivities comparable to that of heavily doped semiconductors [6]. An evaluation of the electrostatic properties of a MT, based on the recent investigation of the structure of tubulin [7], showed an overall negative electrostatic potential of the MT with smaller sub-regions of positive potential [8]. Existence of conduction pathways in MTs based on the electron transfer via hopping or tunneling along electron-rich amino acids (with aromatic groups) within the structure of tubulin was also conjectured [9].

We report on experiments designed to determine the magnitude of the electrical conductivity of MTs. The difficulties associated with the measurement of the electrical resistivity of a single MT lead us to take a macroscopic approach. In our experiments we employed measurements of electrical resistance of a collection of MTs deposited on a silicon substrate with suitable metallization patterns formed on the layer of thermally grown silicon dioxide. Since MTs are not stable outside an aqueous environments with high ionic strength (namely a salt rich buffer solution), we devised a series of control experiments with measurement of the electrical resistance of MT-free solutions (with ultra-pure water and with a buffer solution) and solutions that contain MTs to estimate the electrical resistance of the collection of deposited MTs. From measurements we estimated that the electrical conductivity of a MT cannot exceed the upper limit, which is approximately at the level of 90 Ω^{-1} m^{-1}.

The paper is organized as follows: In ''Experimental methods'' section, we present a detailed description of experiments performed in this study. The measured data was analyzed and essential results are discussed in ''Results and discussion''. Finally, conclusions concerning electrical conduction in MTs are presented in ''Conclusions''.

Experimental methods

Synthesis and stabilization of MTs

In this work, we used low purity MAP-rich tubulin (~30% MAPs). The tubulin preparation was prepared from bovine brain extracts (Cytoskeleton Inc), and was stored at –70 °C in G-PEM buffer (pH 6.8). The G-PEM buffer contained 80 mM Piperazine-*N*, *N*′-bis[2-ethanesulfonic acid sequisodium salt (PIPES), 1 mM magnesium chloride ($MgCl_2$); 1 mM ethylene glycol-bis (*β*-amino-ethyl ether) *N*,*N*,*N*′,*N*′-tetra-acetic acid (EGTA) and 1 mM GTP.

In vitro MT assembly was performed in PEM 80 buffer (80 mM PIPES, 1 mM EGTA, 4 mM $MgCl_2$, using KOH to adjust pH to 6.9) with a final tubulin concentration of 1.5 mg/ml. Polymerization was performed by the addition of GTP (final concentration 0.25 mM). Taxol (~20 μM) was added as a stabilizing agent after polymerization of the MTs was completed. The final solution was rotated at low speed (15 rpm) for 30 min at 37 °C for polymerization. Then, the solution was centrifuged at 1,4500*g* for 30 min to separate MTs from the un-polymerized tubulins. The supernatant was removed and about 5-fold volume of fresh PEM 80 buffer was added to resuspend the MTs. The MTs stock solution was diluted with PEM buffer solution in a ratio 1:25 or 1:50 depending on the experiment performed.

The buffer solution used to stabilize the MTs is a reasonably good electrical conductor, with a measured conductivity of 1.08 Ω^{-1} m^{-1}. Since MTs are expected to have a lower conductivity than the buffer solution, the measurement of their electrical resistance needs to be done in an environment with significantly lower conductivity. Microelectronic grade ultra pure water is the prime candidate to house MTs because of its very poor conductivity (~5.5×10^{-6} Ω^{-1} m^{-1} prior to being exposed to air and approximately one order of magnitude larger when aerated). However, MTs suspended in ultrapure water from stock solution are unstable and disassemble within one minute. In contrast, a fluorescent microscopy pilot study of MTs deposited from stock solution (1:50 dilution) onto Poly-L-lysine (P-L-l) coated glass slides (30 min deposition time) and subsequently rinsed in ultra pure water showed that MTs attached to the P-L-l film survived for approximately 5 min with complete disassembly after 10 min. The

electrostatic interaction between the negatively charged MT and the positively charged P-L-l film is apparently the main factor that slows down the dissociation of tubulin dimers from the polymerized MTs. Indeed, AFM measured disassembly rates of MTs adsorbed on surfaces have been shown to be significantly lower than disassembly rates in solution [10]. In that study, maximum and minimum rates of 17.3 and 0.62 nm/s were reported from the AFM observation of disassembly of MTs electrostatically bound to a surface. From these extremes, we estimate that a median disassembly rate of ~8.6 nm/s is consistent with our own observation of complete disassembly of MTs with an average length of ~5 μm on a P-L–1 film. All the experimental measurements reported in this paper are therefore for MTs stabilized by attachment onto a P-L-l film. We have also verified that P-L-l coated glass slides have negligible electrical conductivity.

Sample geometry

The substrates used in the experiments consist of 4 mm × 4 mm oxidized silicon chips (wafer samples) with large gold square electrodes (100 μm on a side) formed on the layer of oxide (silicon dioxide) and separated by a 10 μm gap (Figs. 1a, 3a). The oxide layer is ~500 nm thick. The gold electrodes rise above the silicon dioxide with a measured step height ranging from 65 to 95 nm. A representative cross section of the wafer with gold electrodes is illustrated in Fig. 1b. The surface of each chip was coated with P-L-l by immersion in a mixture of 600 μl of P-L-l solution (Sigma Diagnostics Inc, P-L-1, 0.1% w/v in water with thimerosal, 0.01%, added as a preservative) and 300 ml of ethanol for 20 min, and then dried at 60 °C. Chips coated with P-L-l were considered good for only 3 days after coating. Fluorescent microscopy of MTs deposited onto a wafer sample and rinsed in ultra pure water showed that P-L-l does not coat the gold metal electrode since MTs survive on the silicon dioxide but disassemble on gold (Fig. 2). The length and surface density of the deposited MTs on the silicon dioxide surface is sufficient to establish electrical contact between the gold electrodes.

Experimental set-up and protocol

Each chip was placed in a Petri dish (diameter of 9 cm, height of 1 cm) which was placed inside a Micromanipulator 6,000 series measuring desk station with a microscope that is required for positioning the probes tips at the center of the gold squares (Fig. 3). Two micromanipulator probes made out of tungsten with 1 μm probe tips were used to access the two gold squares of a wafer. The probes are connected to an Agilent 4155C Semiconductor Parameter Analyzer. The analyzer was set to sweep voltage at medium sweep speed with increments of 0.01 V from –1 to +1 V with maximum allowed current of 10 mA. In order to minimize electromagnetic noise all measurements were conducted in a metal case and coaxial cables were used to connect probes to the analyzer. The resistances of the gold squares, probes, cables and contacts in this set-up are insignificant in comparison to the resistance of aqueous solutions.

The Petri dish is used to immerse the sample in 50 ml of ultra pure water. For the sake of consistency,

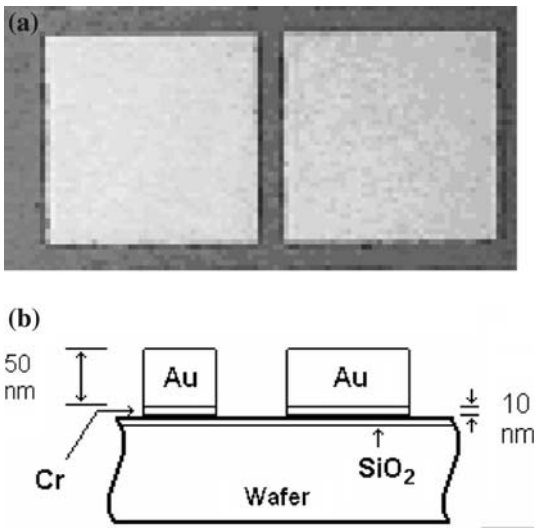

Fig. 1 (**a**) Top view of patterned silicon chip used as a substrate. The light regions are gold and the darker regions are silicon dioxide, (**b**) cross section schematic of gold electrodes patterned patterned on chip. A thin layer of chromium is used to improve adhesion of gold to the substrate (silicon dioxide)

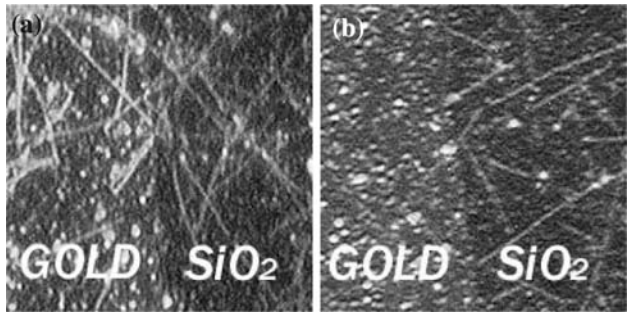

Fig. 2 Fluorescent microscopy pictures of microtubules (MTs) deposited on Poly-L-lysine (P-L-l) coated wafer prior (**a**) and after (**b**) rinsing with ultra pure water for 3 min. The initial MT stock solution had a 1:50 dilution. The length of the MTs range from 3 to 10 μm

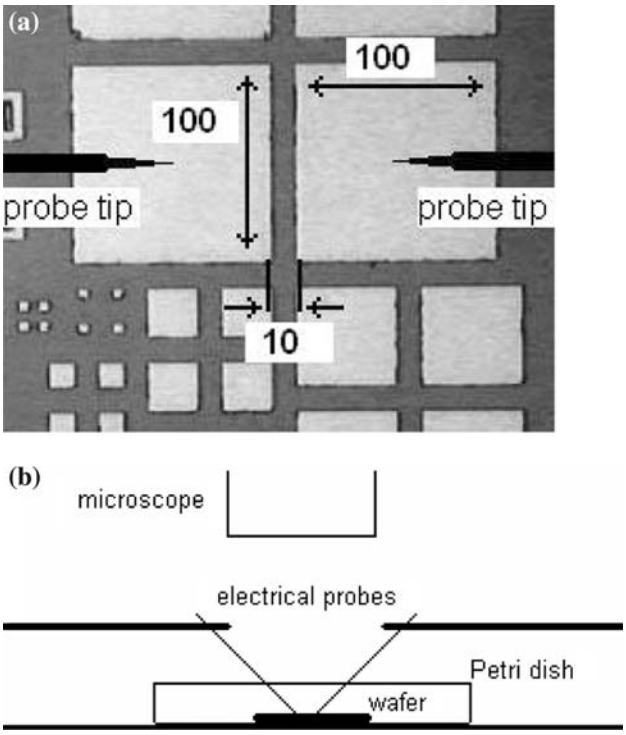

Fig. 3 Top view (**a**) and schematic side view (**b**) of the experimental set up. Dimensions are in micrometers

we employ ultra pure water aerated for 24 h in a clean half-filled container. To avoid contamination, we used ultra clean Petri dishes. The glass surfaces were cleaned with standard microelectronics glass cleaning procedures. Since MTs are dynamically unstable in ultra pure water, all measurements of resistance made in this study are time dependent. The following solutions were utilized in the experiments: (a) 5 μl drops of buffer solution covered with DI water (50 ml), (b) buffer solution with MTs (1:25 dilution), and (c) tubulins in water (same nominal concentration as buffer solution with MTs). In the individual experiments the solutions were placed between the gold electrodes and left to stand for 30 min in an enclosure to avoid evaporation. The sample was then moved into the Petri dish and the probes were placed on the electrodes before addition of water. In each experiment, once the water was poured in, a voltage sweep was performed every 30 s for 20 min. Each measurement consists of a current versus voltage plot having the characteristic form of an electrolytic reaction (current is an exponential function of potential) [11]. The resistance of a sample is determined as the slope of a current versus voltage plot at the equilibrium potential (i.e. zero current). Measurements of the resistance of pure water, buffer solution and non-polymerized tubulin are used as controls.

Results and discussion

Results

We measured the time evolution of the electrical resistance of several samples for each solution (water, buffer, buffer with MTs and water with tubulin). Figure 4 shows the average resistance and its standard deviation as functions of time for these four systems. The average electrical resistance of all the solutions shows a small decrease during the first couple of minutes which we associate with desorption of P-L-l (the only chemical common to all samples) from the oxidized chip surface. The release of some ionic P-L-l results in a higher electrical conductivity of the solutions between the electrodes. These observations were verified by drying different amounts of the P-L-l on the substrate and by using different amounts of P-L-l during the resistance measurements. The solution of tubulin dimers in water mimics the time evolution of the resistance of ultra pure water. This observation indicates that tubulin in un-polymerized form does not affect the electrical conductivity of pure water nor does it affect the electrical conductivity of our samples even if it were to adsorb onto the P-L-l coating between the electrodes. The electrical resistance of samples treated

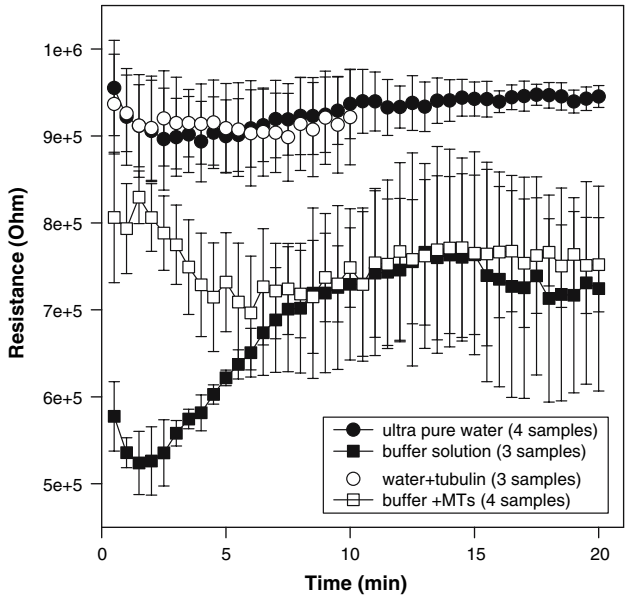

Fig. 4 Time evolution of the average electrical resistance and standard deviation of untreated samples in pure water (filled circles) and samples immersed in a Petri dish containing ultra pure water after deposition of a 5 μl drop of water containing dissociated tubulin (open circles), buffer solution (5 μl) represented by the curve with filled squares, and buffer solution with microtubules (MTs) (open squares). The number of samples studied in each case is indicated in the legend

with the buffer solution exhibits a strong time dependency. Following the brief early decrease in resistance, 2 min after addition of the ultrapure water in the experimental dish, the sample with buffer solution shows the lowest electrical resistance of all the solutions studied. The buffer solution contains a significant number of ionic salts and the presence of the charged chemical species might be a cause of the observed high electrical conductivity of the solution. The average resistance increases steadily for approximately 7 min apparently due to the slow desorption of the charged chemical species from the surface of the channel formed by the two raised electrodes (Fig. 1b). Diffusion out of the ~65 nm deep channel would occur on a time scale of approximately microseconds and cannot account for the observed change in resistance lasting 7 min. After 10 min, the electrical resistance of the samples with the buffer reaches a plateau indicating that desorption may have stopped. However, the electrical resistance of these samples remains below that of the samples with ultra pure water suggesting that some charged species have remained adsorbed onto the surface of the channel providing pathways for conduction between the electrodes. Bulk effects are not believed to be responsible for this lower steady resistance, since we have calculated that the amount of buffer solution used in the experiments (5 μl) is so small that its contribution to the electrical conductivity of the 50 ml of ultra pure water filling the Petri dish is negligible. In reference to the work of Thomson et al. [10], this effect might also be due to the large fluctuations in the disassembly rate of MTs as observed by atomic force microscopy in the referenced publication.

The buffer solution and the buffer solution with MTs have similar chemical compositions. One would expect the resistance of the samples with the buffer and with buffer and MTs to be identical if MTs were poor conductors. If the resistance of MTs were lower than that of the buffer solution, the initial average resistance of the buffer solution with MTs would be lower than that of the buffer solution alone. This observation cannot result from MTs and other associated proteins covering enough of the gold electrode surface to decrease the available conducting area since (a) the resistance of the system with ultra-pure water and of the system with water and dissociated tubulin are comparable and (b) the dissociating rate of MTs adsorbed on gold is very fast which is incompatible with the 7 min necessary for the resistance to approach that of the buffer solution. Complementary measurements of the electrical resistance of bulk buffer and buffer with MT solutions concur with these observations. This implies that the MTs increase the effective resistivity of the entire buffer solution. This may occur because MTs adsorb to their surface or trap within their protein structure charged species in the buffer, thereby inducing a reduction in the conductivity of the buffer solution. This last statement might be difficult to justify and the only evidence to support this is the decrease in resistance of buffer solution with MTs. This difference was determined during the first 5–6 min of the experiments. In addition, in the stability study of MTs deposited onto P-L-l films in contact with pure water it was observed that the steady dissociation of the MTs occurred during this time period (5–6 min). Apparently, release of the trapped charged chemical species during disassembly of the MTs inside the electrode channel improves conduction and consequently the measured electrical resistance decreases. This process dominates the competing slow diffusion of the buffer solution from the channel into the Petri dish. After 6–7 min the samples prepared with buffer solution and MTs have an electrical resistance comparable to that of the samples with the buffer solution and no MTs. As observed previously, dissociated tubulin does not appear to influence the electrical resistance of the samples. The large standard deviations of the buffer solution and of the buffer solution with MTs may be the result of the variability in the process of deposition of MTs inside the electrode channel.

Estimation of the electrical conductivity of a single MT

A lower limit of the electrical resistance of MTs in the samples containing buffer solution and MTs can be estimated assuming that the MTs have adsorbed all the charged species present in the buffer solution. This means that we neglect the effects due to charged species contributed by buffer solution. Consequently the electrical conduction occurs through the background high resistivity medium and MTs. This process can be modeled as a parallel connection of resistor representing the background conduction and the resistor, R_{MTs}, representing the conduction through MTs. The experimentally measured initial resistance of this system, R_{is}, was determined with a value of $8.0 \times 10^5\ \Omega$. The background medium resistance R_{ib} is close to that of pure water with an initial resistance of approximately $9.5 \times 10^5\ \Omega$.

Assuming the proposed model, the MTs resistance, R_{MTs}, can be calculated using the formula for parallel resistors:

$$\frac{1}{R_{\mathrm{is}}} = \frac{A_{\mathrm{f}}}{R_{\mathrm{ib}}} + \frac{1}{R_{\mathrm{MTs}}} \tag{1}$$

Here, A_f is the fraction of the area of the electrode exposed to water. We assume that the MTs are attached to the P-L-l film at the bottom of the trench delimited by the gold electrodes. As seen in Fig. 2b, the network of attached MTs to the P-L-l on the silicon oxide surface will form connections between the electrodes. The electrical resistivity, ρ_{MT} of MTs can be estimated using the equation:

$$\rho_{MT} = R_{MTs}\frac{A}{L} \quad (2)$$

where A is the combined area of MTs making connections between the electrodes and L is the separation distance between the electrode (here 10 μm). Based on fluorescence microscopy analysis of the surface density of MTs deposited on P-L-l films, we have estimated that, prior to the addition of ultra pure water in the Petri dish, there will be approximately 50 connections between the two gold electrodes made of one or several touching MTs. MTs are hollow cylinders, however, to estimate A we use the total area of the cross section of a MT in order to calculate the resistivity of a MT and not of the tubulin protein. With a diameter of 24 nm, the combined area of the MT connections, A, amounts to 2.25×10^{-14} m^2 which is much smaller than the area of the gold electrode. Consequently we can assume that $A_f \sim 1$, and calculate the lower limit for the resistivity using formula (1). This simple calculation yields an estimate of minimum MTs resistivity as 1.1×10^{-2} Ωm (the corresponding electrical conductivity is ~90 Ω^{-1} m^{-1}). With this resistivity, a 10 μm long MT would have the electrical resistance of 240 MΩ, suggesting that a microtubule alone would not be a good candidate for an electrical nano-interconnect (nano-wire).

Conclusions

Using a combination of microelectronics, electrical measurements, and biotechnology, we performed a series of measurements to estimate the electrical resistivity (conductivity) of microtubules. MTs grown in a buffer solution with high ionic strength were deposited on P-L-l coated oxidized silicon chips containing gold electrodes. The time evolution of the electrical resistance of this system immersed in ultra pure water was monitored. From the experimental measurements a clear difference in the conductivity of the solutions in the presence and absence of microtubules was observed. A series of control experiments involving: ultra pure water, ultra pure water with un-polymerized tubulins, buffer solution with and without MTs, were performed to verify and estimate the resistance of the MTs. Since MTs are unstable outside specific buffer solutions, their electrical properties appear to be confounded with the properties of these solutions. The dynamic measurements of the resistance of MTs deposited on the featured silicon chips were used to follow the disassembly of the MTs. Furthermore, we estimated that the upper limit of electrical conductivity of MT is of the order of ~90 Ω^{-1} m^{-1}. This value is comparable to conductivities of inorganic intrinsic semi-conductors at room temperature (which is between 10^{-3} and 10^{3} Ω^{-1} m^{-1}) [12], indicating that MTs are poor conductors.

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