Micron 83 (2016) 11–18

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Review

Characterization of tip size and geometry of the pipettes used in scanning ion conductance microscopy

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ARTICLE INFO

Article history:
Received 14 October 2015
Received in revised form 5 January 2016
Accepted 12 January 2016
Available online 15 January 2016

Keywords:
Nanopipette
Scanning ion conductance microscopy (SICM)
Atomic force microscopy

ABSTRACT

Scanning ion-conductance microscopy (SICM) belongs to the family of scanning-probe microscopies. The spatial resolution of these techniques is limited by the size of the probe. In SICM the probe is a pipette, obtained by heating and pulling a glass capillary tubing. The size of the pipette tip is therefore an important parameter in SICM experiments. However, the characterization of the tip is not a consolidated routine in SICM experimental practice. In addition, potential and limitations of the different methods available for this characterization may not be known to all users. We present an overview of different methods for characterizing size and geometry of the pipette tip, with the aim of collecting and facilitating the use of several pieces of information appeared in the literature in a wide interval of time under different disciplines. In fact, several methods that have been developed for pipettes used in cell physiology can be also fruitfully employed in the characterization of the SICM probes. The overview includes imaging techniques, such as scanning electron microscopy and atomic Force microscopy, and indirect methods, which measure some physical parameter related to the size of the pipette. Examples of these parameters are the electrical resistance of the pipette filled with a saline solution and the surface tension at the pipette tip. We discuss advantages and drawbacks of the methods, which may be helpful in answering a wide range of experimental questions.

Contents

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http://dx.doi.org/10.1016/j.micron.2016.01.002
0968-4328/

1. Introduction

The scanning ion-conductance microscopy (SICM) belongs to the family of scanning-probe microscopies, in which the topography of a sample is reconstructed by scanning a probe over the sample surface, while measuring a physical parameter. In SICM, in particular, a glass pipette filled with an electrolytic solution and containing an Ag/AgCl electrode is used as the probe to map the topography of non-conducting surfaces immersed in a bath of the same solution. A reference electrode is also placed in the bath to close the electrical circuit. In presence of a voltage between the pipette and the bath electrodes, the ion current flowing through the pipette aperture is used to monitor the probe-sample distance (Hansma et al., 1989). In fact, when the volume available for the electrolyte ions flow is decreased, as a consequence of the approach between pipette aperture and underlying surface, the current also decreases. The absence of a direct physical contact between probe and sample during normal operation makes this technique particularly suited for imaging the surface of living cells (Korchev et al., 1997). The addition of a shear-force distance control further expands the potential of SICM, which may be used to map surface conductivity (Bocker et al., 2007).

The shape and size of the probe tip affect the performance of the technique in several ways. First of all, the most important feature of a microscopic technique, i.e. the spatial resolution, is linked to the size of the pipette aperture. Finite element modelling (FEM) simulations have been carried out to investigate SICM lateral resolution. Rheinlaender and Schaeffer and Edwards et al. agreed on a value of the lateral resolution corresponding to about three times the aperture radius (Rheinlaender and Schaffer, 2009; Edwards et al., 2009). Recent experiments suggested that better resolution, equivalent to about one tip radius or even lower, can be achieved (Weber and Baker, 2014). However, Rheinlaender and Schaeffer presented a direct comparison of numerical and experimental data, confirming that the SICM lateral resolution is about three times the aperture radius, for pipettes in a wide size range (Rheinlaender and Schaeffer, 2015).

The angle of the conical tip is also important because it takes part in the pipette resistance. Higher cone angles correspond to lower pipette resistance values, and in turn to higher sensitivity of the probe to the distance (Rheinlaender and Schaffer, 2009; Edwards et al., 2009). Sa and Baker have recently demonstrated that both the aperture radius and the tip cone angle affect the extent of current rectification observed at low values of the electrolyte concentration (Sa and Baker, 2013). The external to internal radius ratio at the tip also affects the shape of the current-distance function: higher values of the ratio correspond to higher sensitivity to the probe-sample separation (Rheinlaender and Schaffer, 2009; Edwards et al., 2009). However, Del Linz et al. have outlined as very often the scanning of a sloped surface results in unwanted contact between the external surface of the pipette tip and the sample under investigation (Del Linz et al., 2014). The knowledge of the external tip radius would allow one to estimate the maximum sample slope that can be imaged without contact.

Several variations of the basic SICM setup have been developed in order to implement specific functions: for example the controlled delivery of molecules with nanometre precision (Babakinejad et al., 2013; Hennig et al., 2015) or the application of controlled mechanical stimuli to cells (Sanchez et al., 2008; Pellegrino et al., 2011). In these cases, the application of a pressure to the pipette back is exploited to generate a liquid flow at the tip aperture. The knowledge of the aperture size is extremely important, because the flow released at a given pressure scales as the fourth power of the tip radius (Schnorf et al., 1994).

The points mentioned above illustrate just a few of the reasons why the geometrical characterization of the SICM probe is very important. According to the literature, SICM experimentalists are used to characterize the pipette mostly by SEM imaging or by measuring electrical resistance. However, cell physiologists started using pipettes before the middle of the latest century and the history of pipette characterization is almost parallel. Thus, we propose in this paper an overview of the literature regarding the measurement of pipette tip, including methods used in SICM and methods used in other fields, which potentially could be useful also to SICM users. Recently, SICM and Scanning ElectroChemical Microscopy (SECM) have been combined by using multi-barrelled pipettes or other kinds of integrated probes (Comstock et al., 2010). In this paper, we limited our scope to methods applied to single barrelled pipettes; however, the same methods (and especially direct methods, see below) can be useful to characterize multi-barrelled pipettes.

Section 2 provides a general description of the pipettes and outlines the features relevant to using them as probes for the SIC microscope. Then, methods used for characterizing pipette size are described in the following sections, grouped in the categories of direct and indirect methods. The direct methods consist in imaging the pipette tip by resorting to some kind of microscopic technique with nanometric resolution, such as electron microscopy and atomic force microscopy (AFM). Their use in pipette characterization is overviewed in Section 3. Due to the diffraction limit for visible light, optical microscopy is not included in the present review. Indirect methods are based on the measurement of a physical quantity that can be related to the pipette size. Here, in Section 4, we take into account methods based on surface tension and electrical resistance. Section 5 is especially focused on the ratio between external and internal radius at the tip, in comparison to the value of the original capillary. We have tried to bring order to the contradictory reports on this topic. In conclusion, advantages and drawbacks of the different methods are discussed.

A more general overview of SICM applications in biology and in material science is out of the scope of the present work. The readers interested in these topics are referred to recently published reviews (Happel et al., 2012; Anariba et al., 2012; Lab et al., 2003; Liu et al., 2013; Schaeffer, 2013; Scheenen and Celikel, 2015).

2. The pipettes used in scanning ion conductance microscopy

The probes used in SICM are prepared directly in the laboratory day by day, using commercially available glass capillary tubing and pipette pullers. The historical process of development of pullers (with horizontal or vertical orientation, with symmetrical or asymmetrical pulling, with or without air jet, etc.) is described in the book by Brown and Flaming (1986). The description of the physical processes occurring during probes fabrication can be found for example in the papers by Purves (1980) and by Huang et al. (2007). Basically, two pipettes are obtained by heating a short segment of a capillary above the glass working point and then separating the two parts by pulling. The capillaries used for this purpose are typically made of soda glass, borosilicate glass or quartz. Soda glass has a low melting point and produces blunt tips that can scan better a sloped surface. The high conductance of this glass is associated with high noise levels. This drawback can be easily solved by coating the filled pipette shank with a siliconizing agent such as Sigmacote (Ogden and Stanfield, 1987). Pipettes of borosilicate glass or quartz exhibit sharp tips and low noise and allow the fabrication of very narrow pipettes for high resolution SICM.

Glass heating is provided by a resistive coil or by a CO_2 laser. The fine control of heating and pulling parameters should allow one to obtain pipettes with reproducible size and geometry. Different parts can be distinguished in a pipette, as sketched in Fig. 1(a):