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Generation of chip based microelectrochemical cell arrays for long-term and high-resolution recording of ionic currents through ion channel proteins

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ABSTRACT

High throughput and a long life-time of the devices are two crucial challenges in planar chip technology for electrophysiological measurements of ionic current recording through ion channel proteins. In this paper, we present a wafer-scale process for the generation of novel arrays of microelectrochemical cells for long-term and high-resolution current recording. At the bottom of each of the cells, which have typically diameters of around 60 μm and volumes of around 30 pL, a nanocrystalline silver/silver chloride secondary electrode is generated for ionic current recording. The top of the cell is closed by a lid containing a small (6–16 μm) opening which connects liquid in the chamber to a contacting liquid on the outside. The processes necessary for manufacturing such a chip through photolithography and wafer-scale bonding have been developed, the resulting structures were characterized and the procedures were optimized. Combining a large surface area of the electrode with a – in relation to the cell size – relatively large amount of silver/silver chloride allows for the recording of DC ionic currents for prolonged periods of time. First measurements were performed where the electrochemical cells were closed by model membranes containing single ion channel proteins. The currents generated by ions passing through these ion channels are reported. These measurements demonstrate the usefulness of the microelectrochemical cell array for long time ionic current recordings at – for these type of measurements – relatively high current levels.

1. Introduction

Ion channels which are embedded in cell membranes play a crucial role in many cellular functions in humans and animals. As they control the flux of ions across the cell membrane they are related to many important physiological functions [1]. For example, the information exchange within the nervous and the cardiovascular system, as well as the metabolism in cells associated to the intestinal or the reproductive system are regulated by a controlled opening and closing of those membrane channels. Accordingly, ion channel dysfunctions are related to many common diseases [2]. Therefore, screening the influences of novel pharmaceutical compounds onto such ion channel proteins is a mandatory requirement for the approval and introduction of such compounds into medical therapy. In the classic patch-clamp technique developed in the seminal work of Neher and Sakmann [3,4], a glass pipette tip is applied in such a way that it connects with the cell membrane and forms a tight electrical seal between the pipette tip and the cell membrane, so that ionic currents flowing across the membrane can be recorded. However, this method is experimentally challenging, time consuming and has very low throughput. To study a large number of compounds with high reliability, a high throughput analytical approach is required.

A planar patch-clamp chip technology has been developed to overcome these problems of the classical patch-clamp technique. To do this, chip-like devices have been developed, which contain a small microaperture integrated in a glass or silicon substrate. The aperture replaces the glass pipette and is used for cell connection and high electrical seal formation [5,6]. In order to further increase throughput, a microelectrode cavity array (MECA) has been

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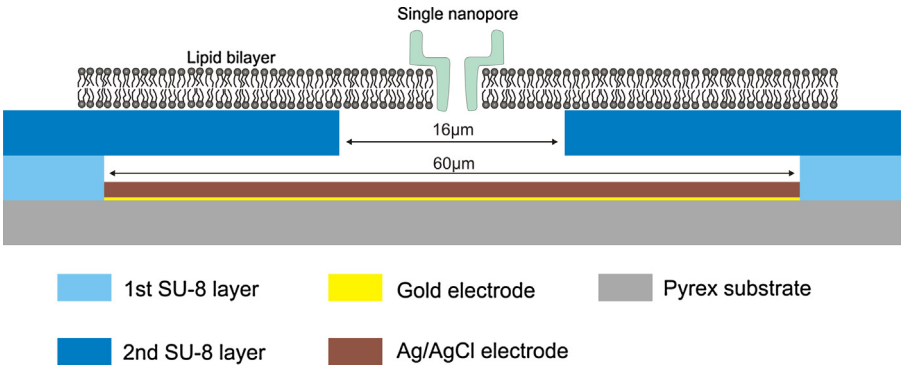

Fig. 1. Schematic depiction of the generated microelectrochemical cells. In this 3D undercut structure, the cavity (⌀60 µm) generated in a SU-8® layer on top of a glass substrate contains a secondary Ag/AgCl microelectrode on top a gold structure. The cavity is closed by a second SU-8® layer containing a microaperture (e.g. ⌀16 µm). The thickness of each SU-8® film is around 10 µm. The cavities are coated with painted lipid bilayers containing single nanopores.

fabricated where the structures were generated using the SU-8® photoresist [7]. In this approach an array of microelectrochemical cells with typical volumes of less than 1 pL is generated, where the apertures at the top of the cells can be sealed by membranes. Silver/silver chloride microelectrodes, which have been integrated into the microcavity structures, allow recording of the electrochemical behavior of ion channels contained in the membranes. In this way, larger numbers (for example 16 in [8]) experiments can be carried out in parallel, so that the throughput of the patch-clamp method is increased by more than an order of magnitude. Both planar patch-clamp chips and MECA chips can be used for recording of the flow of ionic currents through single channel proteins [5,8,9]. In the latter, electrolyte exchange between the two half-cells, which are separated by the membrane and which are connected by the ion channels, is monitored by using a secondary electrode, e.g. a nanocrystalline Ag/AgCl electrode deposited on top of gold microelectrode present in the MECA chip [7].

If the electrophysiological properties of living cells are to be recorded, it is obvious that the size of the opening of the microelectrochemical cell must be smaller than the size of the biological cell to be tested. As the maximum size of the cavity is given, also the electrode size and volume contained in the cavity is strictly limited and typical electrodes consist of only 10^{-12} mol of silver [7]. This, however, poses a significant problem when higher currents are recorded. As in a small piece of cell membrane typically thousands of ion channel proteins are contained, the current flowing across the cell membrane is much higher than that occurring in single channel measurements. This in turn, however, leads to rapid consumption of either the silver or the silver chloride (depending on the current direction) and loss of the functionality of the secondary electrode. As a result, the electrical stability of microelectrode is a critical factor for the performance of electrophysiological studies of biological cells with such planar patch-clamp chips. Increasing simply the chamber diameter (which would allow for larger electrodes with more capacity) is not a viable option as the diameter of the opening must be smaller than the size of the cell. Even when the focus of the measurements is placed on model membranes, increasing the aperture would still not desirable as an increase of the clear span of the self-supporting membrane would strongly compromise the mechanical stability of the system. Therefore, a microelectrochemical cell with a small opening and a high electrochemical capacity is required, which could be achieved by generating a larger cell and sealing it by a lid containing a small aperture, which is essentially the same as the generation of a 3D undercut structure.

A key requirement for the successful construction of such devices is that the Ag/AgCl electrode is already introduced before the aperture is generated as a galvanostatic deposition of a high quality Ag/AgCl electrode after complete assembly of the microelectrochemical cell is no longer possible. In such a case the sealing of the cell with an impermeable lid having only a small hole through which the ions can migrate would lead to a quite strongly inhomogeneous electrical field upon application of a potential [10,11], This would in turn yield very inhomogeneous silver layers in the electroplating process and undesired morphologies of the nanocrystalline silver. On the other side, once the metal is introduced, further lithographic steps cannot be performed easily due to problems with reflections and scattering at the surface of the nanocrystalline silver layer.

Commonly, undercut or suspended microstructures based on negative photoresists can be manufactured by wet or dry etching of a metal sacrificial layer under the patterned resist structures [12], such as Cu and Cr [13,14]. After patterning of the resist, the metal layer is removed by an electrochemical or chemical etching process to form the negative microstructures. Alternative materials for the generation of such sacrificial layers are polymers which can be dissolved in an appropriate solvent [15,16]. Another approach to generate negative microstructures, which avoids the presence of sacrificial material, is the application of a UV-blocking layer [17]. After photolithography processing, a thin metal film (Al or Cr) is deposited by sputtering or electron beam evaporation onto the patterned resist surface. Afterwards, another resist layer is coated onto this metal film and patterned, so that the metal blocks out the UV light to prevent the lower photoresist layer from further crosslinking during the UV exposure of the top resist layer [18].

The use of two layers, which are fused together by solvent or thermal bonding, is also frequently applied to fabricate closed microstructures, especially for the generation of microfluidic devices and microchannels [19]. During the bonding process, polymer chains from the two counterparts will interdigitate or interdiffuse with each other under application of a high bonding pressure. The process can be enhanced by increasing the chain mobility through heating or by lowering the glass transition temperature of the polymer through solvent exposure. In some cases an additional covalent binding of the polymer chains contained in the two work pieces can occur at high temperature during high temperature ('hard bake') processes. Many parameters including surface roughness and waviness, crosslinking level, bonding temperature and pressure, must be considered to avoid undesired deformations of the two components or – sometimes even worse – situations where the layers glued together exhibit areas which show weak or no bonding [20].

In this paper, we present an approach based on standard microprocessing technologies (photolithography and lift-off processes) to manufacture an array of microelectrochemical cells with a microchamber structure containing nanocrystalline Ag/AgCl electrodes. The schematic structure of the cells design is shown in Fig. 1. The cells have a diameter of 60 µm with the gold and silver/silverchloride microelectrode at the bottom. At the top the