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A hydrophilic polymer based microfluidic system with planar patch clamp electrode array for electrophysiological measurement from cells

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ABSTRACT

This paper presents a microfluidic planar patch clamp system based on a hydrophilic polymer poly (ethylene glycol) diacrylate (PEGDA) for whole cell current recording. The whole chip is fabricated by UV-assisted molding method for both microfluidic channel structure and planar electrode partition. This hydrophilic patch clamp chip has demonstrated a relatively high gigaseal success rate of 44% without surface modification compared with PDMS based patch clamp devices. This chip also shows a capability of rapid intracellular and extracellular solution exchange with high stability of gigaseals. The capillary flow kinetic experiments demonstrate that the flow rates of PEGDA microfluidic channels are around two orders of magnitude greater than those for PDMS-glass channels with the same channel dimensions. This hydrophilic polymer based patch clamp chips have significant advantages over current PDMS elastomer based systems such as no need for surface modification, much higher success rate of cell gigaseals and rapid solution exchange with stable cell gigaseals. Our results indicate the potential of these devices to serve as useful tools for pharmaceutical screening and biosensing tasks.

1. Introduction

Ion channels are proven to be important molecular targets for a range of clinical drugs since they play important roles in cell electrophysiology and pathophysiology (Xu et al., 2001; Overington et al., 2006; Priest et al., 2006). The patch-clamp technique has become an accepted standard for studying gating of ion channel currents since its invention by Neher and Sakmann (1976). The traditional patch-clamp system consists of glass micropipette tips with a 1–2 μm pore to measure ionic current through part of cell membrane or entire cell membrane area (Sigworth and Neher, 1980; Hamill et al., 1981). However, conventional pipette-based technology is time and labor intensive, which needs expensive equipment and complex setup (Kyrozis and Reichling, 1995; Fan and Palade, 1998; Sarantopoulos et al., 2004). Moreover, proteomics and drug development need high-throughput screening, which is not possible for the traditional glass micropipette method.

Recently, planar patch clamp devices have been developed to replace glass pipettes with parallel measurements to meet the high-throughput requirements (Fertig et al., 2000, 2002; Lehnert et al., 2002; Boussaoud et al., 2012). The materials used for planar aperture fabrication include silicon (Pantoja et al., 2001), silicon oxide coated silicon nitride membranes (Fertig et al., 2000), silicon holes coated with PECVD deposited oxide (Pantoja et al., 2004), silicon oxide nozzles (Lehnert et al., 2002), glass substrates (Xu et al., 2001; Fertig et al., 2002). Major disadvantage of silicon, silicon oxide or glass chips is high processing cost, especially because defining patch apertures involves expensive equipments in the clean room such as focused ion beam (FIB) system.

A reasonable alternative lies in polymers which have many advantages such as cost-effective and flexible manufacturing (Kiss et al., 2003; Stett et al., 2003; Hutter et al., 2013). Recently, poly(dimethylsiloxane) (PDMS) as a popular material for microfluidic application, has been used for planar patch-clamp fabrication (Klemic et al., 2002, 2005; Seo et al., 2004; Li et al., 2006; Schaffhauser et al., 2011). However, the hydrophobic surface property of PDMS may prevent the establishment of a high electrical resistance seal with cell membrane. The probability of successfully recording for PDMS chip with planar apertures is relatively low ($<15\%$ gigaseal success rate). Moreover, it is very difficult to realize the intracellular solution exchange with a PDMS based patch clamp. Generally, the planar patch clamp seals will be broken if the pressure under seals exceeds 15 mmHg (Li, 2006). The requirement of large external driving pressure for PDMS micro-channels makes it impossible to realize this function.

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Surface modification can be performed to change the surface of PDMS from hydrophobic to hydrophilic such as plasma oxidation treatment (Klemic et al., 2002, 2005), or coating of a non-biofouling polymer (Popat et al., 2003; Xu et al., 2011). However, these methods could not guarantee permanent effects or conformal coating. Therefore, in order to solve these problems, there is a need to use new hydrophilic polymeric materials for both microfluidic channels and patch electrode fabrication.

In this paper, we describe a hydrophilic polymer poly(ethylene glycol) diacrylate (PEGDA) based microfluidic patch clamp array system that enables cell signal recording with rapid intracellular solution exchange. PEGDA is a photo-curable polymeric insulator material, which is used to fabricate micro/nano structures by a UV assisted molding method (Kim and Jeong, 2006; Jeong et al., 2007; Liu et al., 2007). The intrinsically hydrophilic property makes it a good choice for patch aperture partition fabrication to facilitate the formation of cell-gigaseal and rapid solution exchange. The feasibility of this PEGDA based patch clamp system is demonstrated for whole cell current recording with yields comparable to that obtained with micropipettes. Cell electrophysiological signal recordings with intracellular solution exchange and extracellular drug perfusion are also realized.

2. Materials and methods

2.1. Fabrication polymeric patch electrodes

Poly(ethylene glycol) diacrylate (PEGDA, MW=258) was purchased from Sigma-Aldrich. This PEGDA liquid polymer contained 100 ppm monomethyl ether of hydroquinone (MEHQ) as a polymerization inhibitor for storage. Before usage, the MEHQ inhibitor was removed using commercially available inhibitor removing columns (Sigma-Aldrich, catalog number: 306312). The photo-initiator 2,2-dimethoxy-2-phenylacetophenone was added at 0.5% (w/w) based on the monomers. The PEGDA based patch electrode partition was fabricated via the process shown in Fig. S1. A 4 in. ⟨1 0 0⟩ silicon wafer with silicon oxide layers on both sides was used as starting substrates. Then, the oxide layer on one side was patterned to open a square etch window of 400 μm for a 2×2 array. The exposed silicon oxide was removed by a wet etching process using buffered hydrofluoric acid (BHF) and the photoresist was removed by acetone and ethanol. KOH (40 wt%, Aldrich) at temperature of 50 °C was then applied to the wafer to etch inverted pyramid-shaped holes. Etching occurred along the crystal plane to form 54.7° tapered walls terminating in a very sharp tip. Then, the sharp tip pattern was transferred to hard-PDMS (h-PDMS) mold by thermal curing. The h-PDMS prepolymer was prepared by mixing four types of materials, a vinyl PDMS prepolymer (4 g, VDT-731, Gelest), Pt catalyst (9 μL, platinum divinyltetramethyldisloxane, IP6831.1, Gelest Corp.), modulator (0.2 g, 1,3,5,7 tetravinyl-1,3,5,7 tetramethyl cyclotetrasiloxane, SIT-7900, Gelest Corp.) and hydrosilane prepolymer (1 g, HMS-301, Gelest Corp.). After mixing, the h-PDMS prepolymer was poured onto the above silicon master and baked at 80 °C in a vacuum oven for 2 h. The h-PDMS replica with sharp tips was peeled off from the master on the hotplate. Next, the backside of the above h-PDMS replica was tightly adhered onto a transparent and rigid chamber with the array of sharp tips in the center of the open window. The h-PDMS side with sharp tips was covered with a planar glass slide coated with a layer of h-PDMS in order to avoid stress difference between two sides. After pouring the PEGDA polymer solution with photo-initiator onto the h-PDMS tips, the contact between the flexible h-PDMS pyramid tips and the h-PDMS layer on the glass surface could be modulated by inner pressure of the chamber to achieve different aperture sizes. Subsequently, the mixture was exposed by UV light (EXFO UV curing system) supplying adequate energy from both sides. Finally, the patch clamp partition with confined apertures was released from the h-PDMS mold.

2.2. Device assembly

PEGDA layers were individually made from PDMS molds and bonded together by UV-assisted irreversible sealing. Generally, a PDMS mold with designed pattern was fabricated using soft lithography method. PEGDA polymer solution with photo-initiator Irgacure 2959 was drop-dispensed onto the PDMS mold. Then, the assembly was put under an EXFO UV curing system (10 mW/cm^2) for partial photo-polymerization and then cooled down to room temperature. After this step, the PEGDA plates were hard and easy to handle. Then the partially polymerized plates, the Ag/AgCl electrode plate and planar patch clamp partitions were assembled and aligned together and exposed to UV light again to complete the photo-polymerization using the Karl Suss contact aligner. As a result, all the parts are covalently bonded together. The edges of the PEGDA partition could be strengthened with 1:1 epoxy glue to increase the bonding intensity.

2.3. Cell culture

Hela cells were used to investigate this patch clamp system capability. Hela cells were cultured in Dulbecco's modified Eagle medium (DMEM) with 4500 mg/L glucose (Invitrogen) as basic medium supplemented with 5% fetal bovine serum (FBS, Invitrogen) together with penicillin and streptomycin (Invitrogen). They were cultured in a humidified incubator at 37 °C with 5% CO_2/95% air. The culture medium was exchanged three times per week and routine subculturing of confluent cell layer was performed using standard trypsinization (0.05% (w/v)/1.5 mM EDTA) techniques.

2.4. Solution

All chemical solutions were obtained from Sigma-Aldrich. The external solution contained (in mM) 145 NaCl, 5 KCl, 1.8 $CaCl_2$, 1.1 $MgCl_2$, 10 HEPES and 10 glucose, adjusted to pH 7.2 with NaOH. The internal solution contained (in mM) 140 KCl, 1.1 $MgCl_2$, 0.4 $CaCl_2$ and 10 HEPES, adjusted to pH 7.2 with KOH. For intracellular experiments, the intracellular solution contained different Ca^{2+} concentrations. Tetraethylammonium chloride (TEA-Cl), as a K-channel blocker for Hela cells, was added to the external solution by equimolar replacement of NaCl.

2.5. Patch clamp chip recording

Patch clamp measurements were made in the whole cell configuration. All experiments were performed at room temperature. For patch clamp experiments, the PEGDA patch clamp chambers were filled with electrolyte solution to remove all the air-bubbles. Signals from individual patch electrode were then collected through the multiplexer by an EPC-10 patch clamp amplifier (HEKA Electronics Inc., Germany). The signals and seal resistance were monitored with multi-channel data acquisition software Patch Master from HEKA Electronics Inc. Data was collected with a 4 kHz cut-off frequency.

3. Results

3.1. Fabrication of PEGDA microfluidic patch clamp system

The schematic of the integrated microfluidic patch clamp array system was shown in Fig. 1. The whole system was separated by