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A new thin silicon microneedle with an embedded microchannel for deep brain drug infusion

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ABSTRACT

We present the implementation of a new thin silicon microneedle for deep brain drug infusion implemented using a fabrication technology called glass cover on silicon technology (GCoS) that embeds microchannels in silicon substrate. The embedded microchannels are formed by anodically bonding a glass wafer to a silicon wafer with cavities, reflowing glass to partially cover the top of the cavity, and removing the unwanted glass above the silicon substrate. Because no dielectric sealing process is required, long microchannels in mm range can be readily fabricated using GCoS and there is no restriction on the cross-sectional area or shape. In addition, the silicon substrate is directly available for further integrated circuit (IC) processing and consists of embedded microchannels that are transparent from top through the glass. Using GCoS, we have successfully implemented a microneedle designed for drug infusion with point targeting accuracy as well as less damage for small animal experiments. The fabricated microneedles were 5.3 mm in length and as small as 40 μm in thickness and 70 μm in width. Fluidic characterization with a pressure-driven injection system showed linearly decreasing flow rates ranging from 100 to < 50 nl/min. This linearity of the flow rates to input pressure confirms precise control of the low flow rates, which is important in injecting small quantity of drugs. After we verify successful infusions of trypan blue with our microneedles into both 0.9% w/v agarose gel and sacrificed mouse brain, we demonstrate the possibility of drug infusions with not only precise targeting capability but also less brain damage by infusing dyes *in vivo* followed by immunohistochemistry (IHC).

1. Introduction

Microinjection has a wide range of uses in neuroscience as a means to delivery genes, dyes, and viruses to local target regions for experiments *in vivo* and *in vitro* [1,2]. For example, direct injection of drugs to a specific target region in brain facilitates investigation of brain functions and their functional connectivity by stimulating and suppressing neural activities [3]. In addition, direct injection of drugs in animal models is widely used to screen drugs for brain diseases in early development stages because intraperitoneal injection or oral administration are ineffective due to the blood–brain barrier (BBB) [4–6]. The BBB blocks most of drugs injected in the blood from entering the brain extracellular fluids. Furthermore, transgene delivery with accurate spatial targeting by viral transfection is an essential procedure in generating genetically encoded proteins in optogenetics [7].

Current microinjection method using hypodermic needles suffers from large damage and inability to infuse small amount of drugs [5]. Despite the continuous efforts to decrease needle dimension, delivery of drugs to a point target region in deep brain of small animals is still a challenge because of large outlet area. The smallest hypodermic needles that are commercially available is 32 ga which has an outer diameter (OD) of 235 μm while companies offer a customized needle as small as 34 ga with OD of 184 μm for neuro applications at an extra cost [5]. However, the tip of these hypodermic needles is slanted at a large angle to ease insertion and thus the contact area during drug release is inevitably larger than that defined by the cross-sectional inner diameter. For instance, ventral tegmental area (VTA), an important region in brain that governs reward systems, is located in deep brain and is only ~700 μm in

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height and ~350 μm in length for a mouse brain. Point targeting a subsection of such region would be challenging with currently available hypodermic needles.

Microelectromechanical systems (MEMS) microneedles have competitive advantages over conventional needles such as design freedom in placing outlet at the tip and small size to achieve minimally invasive injection. While the traditional needles are susceptible to blockage because the outlet is located at the end of the tip, the MEMS microneedles with outlets placed on the side suffer less from the blockage. Moreover, small size not only reduces tissue damage during the insertion but also allows precise targeting especially in experiments with small animals such as mice. However, the main restriction in further minimizing microneedle dimension is the size of microchannels. Previously, microchannels implemented using various fabrication technologies such as sacrificial layer [8], wafer bonding [9,10], porous silicon [11], and buried channel technology (BCT) [12,13] have been presented (Fig. 1). While sacrificial layer method achieves a transparent cover using a dielectric layer, channel height is limited by the thickness of the sacrificial layer (~ a few μm). In addition, complete removal of the sacrificial layer especially for a long channel (~mm length) is challenging. Microfabrication methods such as porous silicon and BCT are more suitable for miniaturized microfluidic structures because microchannels are embedded in silicon substrate. These methods, however, require either complex fabrication process steps or an additional sealing layer that increases the overall thickness of the structures. Not only reliably sealing long microchannels using deposition is challenging but also small openings designed for successful sealing often constrain width of microchannels (Fig. 1(c)–(e)). Wafer-bonding method, on the other hand, offers well-controlled cavity quality and design freedom in cavity geometry because no sacrificial layer or dielectric sealing process (*i.e.* BCT or porous silicon method) is required. Thus, wafer-bonding method is an attractive method to embed long microchannels. For example, a long embedded microchannel was readily formed using wafer-bonding method to implement a polymer neural probe with drug delivery capability [9]. By utilizing wafer-bonding method, this polymer neural probe achieved not only state-of-art probe thickness of 50 μm but also achieved multiple outlets [9]. However, for silicon-based microneedles, currently available wafer-bonding technology still requires a thick additional layer above a silicon substrate to cover the microchannels (Fig. 1(b)).

We propose a new method called glass cover on silicon (GCoS) that enables embedding microchannels in the substrate without requiring an additional layer regardless of channel width. The proposed GCoS is based on glass reflow process; glass wafer is first bonded to a silicon substrate with pre-defined cavities and then reflowed but only partially to cover the top of the cavities (Fig. 1(f)). Since the cover is buried in the silicon substrate, the bonded glass layer is no longer needed and is consequently removed to reveal the surface of the original silicon substrate. The planarized surface is now available for consequent IC compatible fabrication processes. (The consequent fabrication processes must be performed under operating temperature approximately below 600 °C to prevent glass from reflowing further down the cavity.) Therefore, the proposed GCoS offers advantages of both wafer-bonding method (*i.e.* design freedom in channel dimensions) and dielectric sealing method used in BCT (*i.e.* embedding microchannel in silicon substrate). Other advantages include transparent top for quick observation of fluid and relatively simple processing. To demonstrate these advantages, we use GCoS to implement microneedles with dimensions as small as 70 μm in width and 40 μm in thickness and demonstrate successful delivery of drugs in 0.9% w/v agarose gel, sacrificed mouse brain, and brain of anesthetized mouse *in vivo*. Our proposed technology is a very attractive method to miniaturize microfluidic devices used for bioassays and experimental manipulations of small animals such as microneedles and microdialysis probes.

2. Design concept

We propose a relatively straightforward method (GCoS) to fabricate embedded microchannels in silicon without an additional layer above silicon substrate and with a freedom in channel dimensions. GCoS consists of four steps: (1) cavity definition, (2) wafer bonding, (3) glass reflow, and (4) glass removal (Fig. 2(a)). Microchannels are first defined on a silicon substrate using either a soft or hard mask and cavities of desired height are formed by etching the silicon. As long as the etch selectivity between silicon and a mask material is high, any known silicon etching methods such as deep reactive-ion etching (DRIE), wet etching (*e.g.* potassium hydroxide, KOH), and xenon-difluoride etching can be used to create different cross-sectional shapes such as rectangular and semicircular shapes. After removing the mask material, a thin glass wafer is anodically bonded to the silicon substrate in a vacuum condition [14]. This low cavity pressure (*i.e.* the vacuum condition for anodic bonding) is important in the consequent glass reflow step because trapped air expands in volume at an elevated temperature and prevents glass from reflowing toward the cavities in silicon. The bonded wafer undergoes a heat treatment at a temperature above the softening point at atmospheric pressure. During the heat treatment, glass reflows downwards to cover the cavity. In this step, the vacuum level is important as it determines the force that attracts the softened glass. The reflow temperature and time are critical in GCoS; temperature should be high enough to allow glass to reflow but not too high to prevent complete blockage of the cavities. Finally, the remaining glass on silicon is removed using a chemical mechanical polishing (CMP). GCoS achieves embedded microchannels of various dimensions using only four steps and one mask layer and silicon substrate is directly accessible for consequent IC processes.

3. Fabrication and packaging

3.1. Fabrication of embedded microchannels

The process starts with defining width (5–60 μm) and length of microchannels (3–7 mm) on a silicon wafer using a photoresist. Microchannels with desired height of 30 μm are formed by etching silicon substrate using DRIE; DRIE is chosen based on its high selectivity to photoresist, fast etching time, and vertical sidewall profile. A 100-μm borosilicate glass wafer (Borofloat® 33) and the silicon wafer are then anodically bonded at 900 V at 350 °C under a vacuum condition (pressure < 10^{-5} Torr). After the anodic bonding, we observed the cross-section of the cavity prior to glass reflow. Neither significant deflection of glass layer nor collapse of microstructures inside the cavity was observed [15]. As the softening point for borosilicate glass is 560 °C, the bonded wafer is subject to heat treatment at 750 °C for 2 h 30 min at atmospheric pressure using a rapid thermal annealing (RTA) system. The remaining glass on top of silicon is removed by chemical mechanical polishing.

The amount of reflowed glass to cover the cavities is a critical design parameter in GCoS and is a function of numerous factors such as reflow temperature, time, initial cavity pressure, volume of cavity, and thickness of glass wafer. The steady-state behavior of the reflowed glass can be predicted from the ideal gas law,

$$PV = nRT, \quad (1)$$

where P is the cavity pressure, V is the cavity volume, n is the number of gas moles in the cavity, R is the gas constant, and T is the cavity temperature. As n and R are constants, the initial