A minimally invasive microchip for transdermal injection/sampling applications

Lucanos M. Strambini, Angela Longo, Alessandro Diligenti and Giuseppe Barillaro*

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The design, fabrication, and characterization of a minimally invasive silicon microchip for transdermal injection/sampling applications are reported and discussed. The microchip exploits an array of silicon-dioxide hollow microneedles with density of one million needles cm\(^{-2}\) and lateral size of a few micrometers, protruding from the front-side chip surface for one hundred micrometers, to inject/draw fluids into/from the skin. The microneedles are in connection with independent reservoirs grooved on the back-side of the chip. Insertion experiments of the microchip in skin-like polymers (agarose hydrogels with concentrations of 2\% and 4\% wt) demonstrate that the microneedles successfully withstand penetration without breaking, despite their high density and small size, according to theoretical predictions. Operation of the microchip with different liquids of biomedical interest (deionized water, NaCl solution, and D-glucose solution) at different differential pressures, in the range 10–100 kPa, highlights that the flow-rate through the microneedles is linearly dependent on the pressure-drop, despite the small section area (about 13 \(\mu\)m\(^2\)) of the microneedle bore, and can be finely controlled from a few ml min\(^{-1}\) up to tens of ml min\(^{-1}\). Evaporation (at room temperature) and acceleration (up to 80 g) losses through the microneedles are also investigated to quantify the ability of the chip in storing liquids (drug to be delivered or collected fluid) in the reservoir, and result to be of the order of 70 nl min\(^{-1}\) and 1300 nl min\(^{-1}\), respectively, at atmospheric pressure and room temperature.

Introduction

One of today’s most attractive applications of microtechnology concerns the development of new diagnostic and therapeutic tools for the biomedical field. Microfabrication is at present pushed to the development of a miniaturized laboratory (Lab-on-Chip, LoC) that can stimulate, extract fluid from, sense, and deliver drugs to biological systems.\(^1,2\)

In recent years, in-plane and out-of-plane, solid and hollow microneedles\(^3-5\) have been intensively investigated for a number of transdermal applications, ranging from nanomedicine\(^6\) to biosensing,\(^7\) due to their ability to penetrate the outermost layers of the skin avoiding pain, limiting infections, increasing effectiveness, and reducing volume during drug administration and fluid extraction, as well as due to their point-of-care potential. Microneedles have been so far demonstrated as a viable route for minimally invasive therapeutic delivery of drugs, such as vaccines,\(^8\) insulin,\(^9\) hormones,\(^10\) and other pharmacetical agents, as well as for rapid detection of physiological information of high clinical relevance from transdermal fluid, such as glucose level\(^11\) and disease biomarkers.\(^12\)

LoC systems that need to interact with the skin in order to accomplish specific tasks, such as extraction of analytes, delivery of therapeutic entities, monitoring of physiological signals, might take great advantage from microneedles, which once incorporated into a Lab-on-Chip would allow interaction with the skin to be performed in non-invasive and pain-free manner, whether diagnostic or therapeutic in nature. Despite the different materials (metal, silicon, polymer, glass, \textit{etc.}), features (solid, hollow, in-plane, out-of-plane), and fabrication processes (LIGA, micromachining, micromolding, \textit{etc.}) reported so far in the literature, microneedles have been mostly used as standalone devices. Integration of microneedles into a more complex microsystem has been demonstrated only in a few cases\(^13-16\).

In this work, we report and discuss the design, fabrication, insertion test, and fluidic characterization of a minimally invasive silicon microchip for transdermal injection/sampling applications. The microchip exploits out-of-plane hollow microneedles featuring a density of one million needles cm\(^{-2}\) and size of a few micrometers, which are at least two orders of magnitude higher and one order of magnitude smaller, respectively, than those usually reported in the state-of-the-art literature.\(^3-16\) The microchip is intended to pierce the skin by means of its micron-sized high-density hollow silicon-dioxide microneedles that are protruding for one hundred microns from the chip surface. The microneedles are in connection with a number of reservoirs.
integrated on the back-side of the chip, which are used to store liquids, extracted from or to be injected in the outermost layers of the skin. Although the microchip fabrication is here demonstrated for a specific case of microneedle array and reservoir, high flexibility is achievable in terms of needle density, size, and length, as well as reservoir number, size, and volume. Insertion tests of the microchip in skin-like polymers, namely agarose hydrogels with concentrations of 2% and 4% wt, are carried out to verify the ability of such high density, tiny microneedles to withstand mechanical forces acting on the needles during insertion without breaking. Fluidic characterization of the microchip is performed with different liquids of biomedical interest (deionized water, NaCl solution, and D-glucose solution) in order to investigate the relationship between flow-rate through the microneedle array and pressure-drop, the latter being in the range 10–100 kPa. Evaporation (at room temperature) and acceleration (up to 80 g) losses through the microneedle array are also investigated so as to quantify the ability of the chip in storing liquids (drug to be delivered or collected fluid) in the reservoir.

This work represents a first step towards integration of microneedles into a more complex microsystem, the latter provided with micropumps and microelectrodes in the reservoir, aimed at the fabrication of point-of-care LoC featuring minimally invasive interaction with the skin for both drug delivery and biosensing transdermal applications. The final, future goal is the realization of an on-chip artificial pancreas exploiting transdermal, minimally invasive microneedles for continuous sampling of interstitial fluids, measurement of the glucose level, and release of insulin to regulate the amount of glucose in the blood.

Microchip design and fabrication

The microchip consists of: i) a microstructured silicon die integrating, on the front-side, an array of hollow silicon-dioxide microneedles, which are designed to penetrate the outermost layers of the skin and inject/draw suitable fluids, in communication with one or more reservoirs, integrated on the back-side, which are designed to store fluids to-be-delivered/drawn throughout the microneedles; ii) a plastic cover, which is bonded to the back-side of the microstructured silicon die, provided with fitting ports that allow loading/unloading of fluids to-be-delivered/collected throughout the microneedles to be performed. Fig. 1 shows a schematic representation (not to scale) of the proposed microchip.

The microchip fabrication is carried out according to two main phases: 1) microneedle fabrication, on the front-side of the silicon die; 2) reservoir fabrication, on the back-side of the silicon die. The main technological steps of both phases are sketched in Fig. 2 and here below detailed with specific reference to a chip containing, on the front-side, an 0.5 cm × 0.5 cm array of hollow microneedles with period of 10 μm (density of 1 × 10^6 needles cm⁻²) and protruding length of 100 μm; on the back-side, 14 independent reservoirs with a volume of about 0.3 μl each, in connection to the microneedle array.

Microneedle fabrication

The starting material is an n-type silicon wafer with resistivity of 3–8 Ω cm, 650 μm thick, (100) oriented, single-side polished. A thermal dry oxidation (1 h at 1050 °C) is carried out to grow a 100-nm-thick silicon-dioxide layer. A 0.5 cm × 0.5 cm lattice of square holes, with side of 4 μm and pitch of 10 μm, is defined onto the front-side silicon-dioxide layer by means of standard lithography (first mask) and buffered hydrofluoric acid (BHF) etching (Fig. 2a). A potassium hydroxide (KOH) etching (17% weight at 50 °C for 20 min) is used to transfer the pattern from the silicon-dioxide layer onto the silicon surface and produce a regular array of pyramidal notches in the position where microneedles are desired (Fig. 2b). The silicon-dioxide layer is removed by means of a further BHF etching. Back-side illumination electrochemical etching (BIEE), 4 h at constant temperature of 22 °C, in an aqueous solution containing 5% (vol) of hydrofluoric acid (HF) is used to produce, from the pyramidal notches, an array of regular and deep macropores with diameter of about 4 μm and depth of about 200 μm. The array of macropores represents the scaffold from which microneedles are obtained. A thermal wet-oxidation (5 h at 1050 °C) is performed to partially convert silicon to silicon-dioxide and obtain from the macropores an array of 1-μm-thick silicon-dioxide micropipes embedded into the silicon substrate (Fig. 2d). Mechanical polishing is performed to remove the 1-μm-thick silicon-dioxide layer from the front-side surface and expose the silicon between adjacent silicon-dioxide micropipes. A further KOH etching (10% weight at 70 °C for 4 h) is used to obtain an array of microneedles with protruding length of about 100 μm (Fig. 2e). This first phase ends by rinsing the samples in pentane and drying them on a hot plate at 50 °C.

Fig. 3(a–c) shows Scanning Electron Microscope (SEM) images (at different magnifications) of the front-side of the silicon chip at the end of the microneedle fabrication phase. SEM images (a–c) refer to silicon dioxide hollow microneedles with an inner side of 3.6 μm, outer side of about 5.6 μm, period of 10 μm, and protruding length of about 100 μm. As it can be seen in Fig. 3(a–c), both a good lateral (parallel to the wafer surface, top view in Fig. 3a and bird view in Fig. 3b) and vertical (perpendicular to the wafer surface, cross-section in Fig. 3c) uniformity of the needles is achieved.

The key role on the microneedle fabrication is played by BIEE of silicon in aqueous HF-based electrolytes, which is employed for producing an array of regular and deep macropores.¹⁷ BIEE

![Fig. 1](image-url) A schematic representation of the microchip for transdermal injection/drawing applications, showing the microneedles, integrated on the front-side of the chip, in connection with the reservoir, integrated on back-side of the chip. A cover applied on the back-side of the chip is also shown.

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is a highly versatile micromachining technique that allows silicon microstructuring to be performed with high flexibility and low cost.18,19 Given the resistivity of the silicon substrate, the diameter $d$ of etched pores (supposed cylindrical) is linked to the pitch $p$ of the pattern and to the etching current density $J$ by the equation $d \sim p \sqrt{(4/\pi)(J/J_{ps})}$, where $J_{ps}$ is the electropolishing current density for flat silicon electrodes.20,21 The height $h$ of etched macropores, for a given $J_{ps}$ value, depends on the etching time.21,22 In this work, an array of macropores with pitch of 10 $\mu$m are produced using an initial etching current density $J_0 = 6.9$ mA cm$^{-2}$ (electrode area exposed to the electrolyte about 0.785 mm$^2$), which is linearly decreased as the etching progresses, with respect to its initial value $J_0$, in order to obtain macropores with a constant size of about 4 $\mu$m over the whole depth of 200 $\mu$m.21,22 The etching voltage is set to a constant value of 1.2 V.

Although microneedle fabrication is here described for a specific set of parameters $d$, $p$, and $h$, arrays of microneedles with diameter ranging from 1–10 $\mu$m, pitch from 4 –20 $\mu$m, and height up to 500 $\mu$m can be fabricated by changing the pattern of the first lithographic mask and tuning the BIEE parameters so as to produce arrays of macropores, from which the microneedles are obtained, with proper geometrical features.23 Moreover, the microneedle thickness can be changed from a few hundred nanometers up to a few micrometers by properly tuning the thermal wet-oxidation step, in terms of oxidation temperature and time.

The microneedle fabrication is similar to the work reported in ref. 24, but with the chief difference of producing silicon-dioxide microneedles protruding from the front-side of the chip, opposite to microneedles protruding from the back-side in ref. 24, thus enabling higher flexibility in terms of reservoir integration on the same chip.

**Reservoir fabrication**

A triangular lattice of 14 square windows with side of 1000 $\mu$m each and pitch of 1400 $\mu$m is defined in the 1-$\mu$m-thick silicon-dioxide layer still present on the back-side surface of the silicon.
die by standard lithography (second mask) and BHF etching (Fig. 2f). A tetramethyl ammonium hydroxide (TMAH) etching (10% weight at 95 °C for 7 h) is performed to obtain 450-μm-deep trapezoidal groove acting as independent reservoirs with a volume of 0.3 μl each (Fig. 2g). The reservoir depth (450 μm) is chosen so as to reach the bottom of the silicon-dioxide microneedles that are embedded into the silicon substrate. A BHF etching is performed to remove the silicon-dioxide cap at the bottom of the microneedles and put them in connection with the reservoirs (Fig. 2h). The microchip fabrication is completed by bonding a plastic cover on the back-side of the silicon die (Fig. 2i).

Fig. 3d shows SEM images (at different magnifications) of part of one of the reservoirs integrated on the back-side of the silicon chip highlighting the outlet of the microneedles in the reservoir.

Fig. 3 SEM pictures of the microchip front-side and back-side after microneedle and reservoir fabrication is completed: (a) top view, (b) bird view, and (c) cross section (at different magnifications) of silicon dioxide hollow microneedles with an inner side of 3.6 μm, outer side of 5.6 μm, period of 10 μm, and protruding length of about 100 μm integrated on the front-side of the silicon chip; (d) top view (at different magnifications) of part of one of the reservoirs integrated on the back-side of the silicon chip highlighting the outlet of the microneedles in the reservoir.

die by standard lithography (second mask) and BHF etching (Fig. 2f). A tetramethyl ammonium hydroxide (TMAH) etching (10% weight at 95 °C for 7 h) is performed to obtain 450-μm-deep trapezoidal groove acting as independent reservoirs with a volume of 0.3 μl each (Fig. 2g). The reservoir depth (450 μm) is chosen so as to reach the bottom of the silicon-dioxide microneedles that are embedded into the silicon substrate. A BHF etching is performed to remove the silicon-dioxide cap at the bottom of the microneedles and put them in connection with the reservoirs (Fig. 2h). The microchip fabrication is completed by bonding a plastic cover on the back-side of the silicon die (Fig. 2i).

Fig. 3d shows SEM images (at different magnifications) of one of the reservoirs integrated on the back-side of the silicon die in connection with the microneedles on the front-side. The silicon-dioxide needles, partly embedded into the silicon substrate, are clearly opened at their bottom and, thus, in connection with the reservoir.

Although reservoir fabrication is here described for the specific case of multiple small reservoirs, it is straightforward to modify reservoir volume and shape by properly changing the pattern of the second lithographic mask of the process flow. For instance, a single larger reservoir can be easily obtained by defining a square window of proper side during the second lithographic step. If the reservoir volume needs to be further increased, the flat cover can be replaced with a cover featuring a cavity of suitable volume.

Fig. 4 shows front-side (a) and back-side (b) optical images of one of the fabricated microchips.

Microchip characterization and discussion

**Insertion tests in skin-like polymers**

A chief requirement of the proposed silicon microchip concerns the ability of microneedles to penetrate the outermost layers of the skin for their full length without breaking during skin insertion. In fact, despite the different materials and technologies developed so far for the fabrication of microneedles for transdermal applications, only few works deal with needles featuring both density and size values that are comparable to those of the microneedles reported in this work. As a consequence, skin penetration tests reported in the literature...
usually concern arrays of microneedles that feature density at least two orders of magnitude lower and size at least one order of magnitude higher than the ones reported in this work. As far as we are aware, insertion of microneedles featuring both density and size comparable with those of this work has not been investigated so far.

In a first phase, a quantitative theoretical analysis of the main forces acting on the microneedles during skin insertion is carried out as a function of the needle geometrical parameters, i.e. external size, thickness, and length, in order to evaluate and compare the maximum value of such forces with the force needed for piercing the human skin. In a second phase, different micromachining processes for needles protruding from the front-side of the silicon die for one hundred microns are employed to perform insertion experiments in skin-like polymers, such as agarose hydrogels commonly used in the literature as a skin model for several applications,29 in order to evaluate the mechanical behaviour of the microneedles, in terms of breakage and insertion, upon application of an external force.

As to the main forces acting on microneedles during skin insertion, maximum values of compressive, buckling, free-bending, constrained bending, and shear forces that the needles can withstand without breaking must be taken into account and compared to the value of the piercing force $P_{\text{piercing}} = P_{\text{piercing, } A}$ that is needed for penetrating the skin, being $P_{\text{piercing}} = 3.18 \text{ MPa}$ the pressure value required for breaking the human skin 30 and $A$ the needle cross-sectional area. Once the needle has pierced the skin, the force needed for further inserting the needle into the skin for its full length is $P_{\text{post-piercing}} = P_{\text{post-piercing, } A}$, where the value of the post-piercing pressure is $P_{\text{post-piercing}} = 1.6 \text{ MPa}$, that is reduced with respect to $P_{\text{piercing}}$. Therefore, if the maximum values of the forces that microneedles can withstand during skin insertion are sufficiently greater than the value of the piercing force, the microneedles will be strong enough to withstand skin penetration without breaking.

Theoretical analysis of the forces acting on the microneedles during skin insertion is carried out by taking into account mechanical properties of silicon dioxide, which is the material the needles are made of. This is considered to be isotropic with Young modulus $E = 73 \text{ GPa}$ and Yield strength $Y = 8.4 \text{ GPa}$, under the hypothesis of elastic deformation of needles for which a linear relationship between stress $\sigma$ and strain $\varepsilon$ ($\sigma = E\varepsilon$; Hook's law) can be retained applicable, for stress values below the $Y$ value. Under the above-mentioned hypotheses, for a given set of needle geometrical parameters, the maximum values of bending and buckling forces that the needles can withstand without breaking are the most critical among the six different loading conditions considered above.31 Therefore, if such values are sufficiently greater than the value of the piercing force, the needles will be strong enough to successfully withstand skin penetration. Moreover, for a given set of needle geometrical parameters (i.e. external size, thickness, and length), the maximum values of buckling and bending forces that the needles can withstand is lower for microneedles with a square cross-section, than for needles with rectangular and circular cross-sections.31 Though the fabricated needles feature a square-like cross-section, which is intermediate between square and circular (see Fig. 3), theoretical calculations are carried out on needles with a square cross-section, in order to perform a worst-case analysis.

The maximum theoretical value of the buckling force that a single needle can withstand during skin insertion, due to misalignment of the applied force with respect to the symmetry axis of the needle, calculated by modelling the base of the needle as a fixed joint and the needle tip in contact with the skin as a pivoted slider, is:

$$F_{\text{buck_max}} = (C \cdot \pi^2 \cdot E \cdot l) l^2$$

$C = 0.25$ being a constant whose value depends on the end condition of the needle, $I = (d_e^4 - d_i^4)/12$ the moment of inertia of square needles with external and internal side $d_e$ and $d_i$, respectively, and $l$ the length of the needle.

The maximum theoretical value of the free-bending force that a single needle can withstand during skin insertion, due to the bending moment generated by lateral movements between the tissue and the needle occurring at the very beginning of the insertion, calculated by modelling the needle as a cantilever beam, is:

$$F_{\text{bend_max}} = (Y \cdot l) (c^2 \cdot l)$$

being $c = d/2$ is the distance of the neutral axis to the outermost edge of the needle.

Fig. 5 shows theoretical values of $F_{\text{buck_max}}$, $F_{\text{bend_max}}$ and $F_{\text{piercing}}$ for a single, hollow silicon-dioxide microneedle with square cross-section featuring a constant silicon-dioxide thickness $t = (d_e - d_i)/2 = 1 \mu\text{m}$, according to the fabricated needles, as a function of the needle length $l$ (Fig. 5(a)) and of the needle external side $d_e$ (Fig. 5(b)). Fig. 5(a) shows $F_{\text{buck_max}}$, $F_{\text{bend_max}}$ and $F_{\text{piercing}}$ for needles with $d_e = 5.6 \mu\text{m}$ and $t = 1 \mu\text{m}$, which is the case of the needles of Fig. 3, as a function of the needle length $l$. It can be seen that the theoretical values of the maximum buckling and bending forces that the needle can withstand without breaking during skin insertion monotonically decrease as the length of the needle increases, thus indicating that the needle becomes more fragile for increasing length. However, for length of 100 $\mu\text{m}$, which is the case of the needles fabricated in this work, the values of $F_{\text{buck_max}}$ and $F_{\text{bend_max}}$ are more than one order of magnitude higher than the value of $F_{\text{piercing}}$ that is required for penetrating the skin, thus ensuring that the
F of significantly depends on the agarose concentration, ranging from some mechanical behaviour of the needles during penetration. Agarose fabricated microchips in order to experimentally evaluate the concentration of 2% and 4% wt, are performed using several distance between the values of $F_{\text{buck max}}$, which is the case of the needles of Fig. 3, as a function of the needle external side $d_e$ (b).

Fig. 5 Theoretical values of $F_{\text{back max}}$, $F_{\text{bend max}}$ and $F_{\text{piercing}}$ for a single, hollow silicon-dioxide microneedle with square cross-section featuring a constant silicon dioxide thickness $t = 1 \mu m$, as a function of the needle length $l$ (a) and of the needle external side $d_e$ (b).

fabricated needles, though very tiny, are able to withstand skin penetration, at least from a theoretical point of view. Fig. 5(b) shows $F_{\text{buck max}}$, $F_{\text{bend max}}$ and $F_{\text{piercing}}$ for needles with $l = 100 \mu m$ and $t = 1 \mu m$, which is the case of the needles of Fig. 3, as a function of the needle external side $d_e$. It can be seen that the distance between the values of $F_{\text{back max}}$, $F_{\text{bend max}}$ and the value of $F_{\text{piercing}}$ monotonically increases as the external side of the needle increases, thus meaning that the needle becomes more robust for increasing external side. From a theoretical point of view, silicon-dioxide microneedles with external side greater than 4 $\mu m$, length of 100 $\mu m$, and thickness of 1 $\mu m$ are robust enough to withstand skin insertion without breaking.

Insertion tests in skin-like polymers, i.e. agarose hydrogels at concentrations of 2% and 4% wt, are performed using several fabricated microchips in order to experimentally evaluate the mechanical behaviour of the needles during penetration. Agarose hydrogels feature a compressive Young modulus that significantly depends on the agarose concentration, ranging from some kPa at concentrations of a few percents up to hundreds of kPa at concentrations of tens percent. Therefore, agarose gels with concentrations of 2% and 4% wt can be effectively used as a first-approximation skin model with the aim of evaluating the mechanical behaviour of microneedles during skin penetration, though further ex vivo and in vivo penetration experiments will be necessary to obtain quantitative information on skin penetration of such high density and tiny microneedles, due to the fairly different Young moduli of the different layers of the skin, in particular of the stratum corneum.

Agarose gels are prepared by dispersing agarose powder in concentrations of 2% and 4% wt in 20 ml of deionized boiling water (100 °C) and stirring the solution for 1 h. The solution is then put into a glass Petri dish with diameter of 9 cm and gelation is completed by letting the solution cool down to room temperature. The resulting hydrogel film, with external diameter of 9 cm and thickness of about 2 mm, is stored overnight at $\approx 5$ °C before using it.

Insertion tests on agarose hydrogels are performed by placing the microchip, with the needles pointing downward, at the bottom of an aluminium cylinder on top of which a constant (200 gf = 1.96 N and 500 gf = 4.90 N), compressive force is applied for different times (30 s, 60 s, and 120 s). The experimental setup only allows vertical displacement of the aluminium cylinder upon the applied force, so that an axial, compressive external force is applied to the microchip in contact through the microneedles with the agarose hydrogel, this latter placed on the bottom surface beneath the cylinder containing the chip. It is worth noting that 500 gf is roughly the typical force value that can be produced through the use of a single human finger. A large set of insertion tests is performed, according to the above-described protocol, using different microchips and agarose hydrogel films. After insertion tests, agarose hydrogel films are analyzed by optical microscopy and microneedles are investigated by SEM observation.

Experimental results show that the proposed silicon-dioxide microneedles can effectively withstand repeated insertion in skin-like polymers without breaking, in agreement with theoretical predictions. Fig. 6(a) shows an optical microscope top-view (magnification of 450 $\times$) of the typical surface morphology of agarose hydrogels with a concentration of 2% wt after indentation with microchips featuring needles with a spatial period of 10 $\mu m$ and side of 5.6 $\mu m$, upon application of 500 gf for 60 s. It can be clearly seen that, despite the very high density of the microneedle arrays, each single needle of the array successfully penetrates the agarose hydrogel, thus producing a nice periodic two-dimensional pattern on the agarose surface. Moreover, no significant damage of the microneedle arrays after insertion tests is noticeable by SEM observation, even for repeated insertion tests performed using the same microchip (Fig. 6(b)). Similar findings are obtained for both types of prepared hydrogels, with agarose concentration of 2% and 4%.
Diagonal indicating one standard deviation, are reported for each tested respectively. In Fig. 7 flow-rate mean values, with error bars indicating one standard deviation for each tested differential-pressure value. Solid lines represent the best-fitting of experimental data using a linear function.

linear relationship (solid lines in Fig. 7) between $F_a$ and $\Delta P$ for all the tested liquids ($R^2$ values ranging from 0.99967–0.99993), which indicates that laminar flow takes place in such tiny microneedles, at least in the differential pressure range investigated, according to the literature. Under the hypothesis that the flow-rate $F_a$ of the whole microneedle array is due to the contribution of $N$ independent and identical needles subjected to the same pressure-drop $\Delta P$, estimation of the flow-rate $F_i$ through a single needle as a function of the pressure-drop $\Delta P$ (see Fig. 5) is performed by using the Hagen-Poiseuille law:

$$ F_i = \frac{s(2d_i^2/\mu C_{ir})\Delta P}{l} $$

where $F_i$ is the flow-rate, as a function of the pressure-drop $\Delta P$, through a single needle with section area, length, and inner side of the needle $s$, $l$, and $d_i$, respectively, $\mu$ is the dynamic viscosity, $C_{ir}$ is the friction coefficient whose value depends on the needle geometry, and $z$ is the slope value of best-fitting curve in Fig. 7. According to the Hagen-Poiseuille law eqn (1) the different dynamic viscosity values of the tested liquids give rise to different values for the curve slope $z$, more specifically 140.34 $\mu l$ min$^{-1}$ kPa, 78.86 $\mu l$ min$^{-1}$ kPa, and 54.11 $\mu l$ min$^{-1}$ kPa for DIW, NaCl solution, and D-glucose solution, respectively. The more the viscosity value of the tested solution decreases the more the slope value of the linear relationship between flow-rate and pressure drop increases, indicating that a higher flow-rate occurs through the microneedles for a given pressure-drop value. The ratio between curve slope values for the three tested liquids agrees quite well with the inverse of the ratio between dynamic viscosity values, as theoretically expected.

By combining eqn (1) and eqn (2), the number of active needles of the array can be estimated by means of the following equation:

$$ N = \frac{z\mu C_{ir}}{s(2d_i^2)}, $$

where SEM observations allow to approximate $C_{ir}$, $d_i$, and $s$ with 57 (needles with square-like inner section), 3.6 $\mu$m (average inner conditions.

**Fluidic characterization**

An experimental fluidic characterization of the microchip is carried out to investigate the relationship between flow-rate $F$ and pressure-drop $\Delta P$, for different liquids of biomedical interest. In particular, a $\Delta P$ range between 10–100 kPa is explored using deionized water (DIW) as well as aqueous solutions of NaCl (4 mol kg$^{-1}$) and D-glucose (1.5 mol kg$^{-1}$), which have dynamic viscosities of 1.0, 1.5, and 2.24 mPa s at the temperature of 20 °C, respectively. The viscosity values are measured by means of a standard viscosimeter and are in good agreement with values reported in the literature. 34

Fig. 7 summarizes experimental flow-rate $F$ data as a function of pressure-drop $\Delta P$ values for the three tested liquids, for both the whole microneedle array $F_a$ and the single microneedle $F_i$, respectively. In Fig. 7 flow-rate mean values, with error bars indicating one standard deviation, are reported for each tested $\Delta P$ value. Best fitting of experimental data yields a very good agreement with values reported in the literature. 34
side of the needles), and \(3.6 \times 3.6 \mu m^2\), respectively. From eqn (3), the number of active needles of the array is about 38 000, in good agreement with SEM observations. In fact, not all the needles of the array on the front-side of the silicon die are in communication with the reservoirs grooved in the back-side of the silicon die, due to the distance of 900 \(\mu m\) between adjacent reservoirs at the bottom. This is a consequence of TMAH anisotropic etching for reservoir fabrication that reduces the reservoir section area from 1000 \(\mu m \times 1000 \mu m\) on top to 500 \(\mu m \times 500 \mu m\) at the bottom. Nonetheless, the total section area of the active needles is still about 1 \(mm^2\), which is eight times greater than the section of a typical hypodermal needle, whose length is, however, much greater, for instance 3 cm, so that liquid injection should be more effective with microneedles for a given pressure drop value, at least from a fluidic point of view.

Control measurements are performed at \(\Delta P = 50\) kPa using DIW, before and after each measurement session with a given liquid in the whole \(\Delta P\) range, in order to monitor partial occlusion of the needle array due to the presence of dust particles with micrometer-size in the liquids. Fig. 8 shows flow-rate average values of each control measurement, with error bars representing one standard deviation. As can be seen in Fig. 8, control measurements show a fairly constant flow rate, with an average value over the whole set of control measurements of 7010 \(l/min\) (solid line in Fig. 8). A maximum percentage deviation (worst case) of 3.5\%, with respect to the above mentioned average value, ensures that no significant obstruction effects occur during flow rate measurements, as it is also confirmed by optical microscope observation of the chip reservoirs after the whole set of measurements.

The microchip fluidic characterization is carried out by means of the flow-meter sketched in Fig. 9, which allows the flow-rate \(F\) with a percentage error of the order of 0.1\%. The flow-meter operating principle is based on the measure of the time interval \(\Delta t\) needed for the flow of a fixed volume \(\Delta V\) of liquid substance, upon the effect of a known differential pressure \(\Delta P\). The ratio between the known volume \(\Delta V\) and the measured time interval \(\Delta t\) gives the liquid substance flow-rate \(F\), that is \(F = \Delta V/\Delta t\). The operating principle is implemented in a pipe of diameter \(D = 0.8\) cm and graduated by ten couples of electrodes put at a regular distance \(L = 2\) cm along the pipe itself. The microchip is put at the bottom of the graduated pipe, with the needles pointing outward. The fluid level in the pipe is monitored as a function of time by measuring the resistance value between each couple of electrodes through a source measure unit (SMU, 2400 SourceMeter, Keithley) connected with a switch system unit (SSU, 7001 Switch System, Keithley). The SSU consists of a set of individually available switches that allows a specific couple of electrodes to be connected to the SMU for resistance measurement at a given time. When the fluid level in the pipe is between the \(i\)-th and \(j\)-th couple of electrodes, the \(i\)-th couple as well as all the electrode couples above it are in an open-circuit condition, which means that their resistance value is ideally infinite and, in any case, exceeding the full-scale range of the SMU; on the other hand, the \(j\)-th couple as well as all the electrode couples below it are in a close-circuit condition, which means that their resistance value is finite and therefore within the measurement range of the SMU. Each time the liquid level goes below a specific couple of electrodes its resistance value exceeds the SMU full-scale range so that a trigger signal is provided. By measuring the time between successive trigger signals obtained from two consecutive electrode couples, for example \(t_{ij}\) for the \(i\)-th and \(j\)-th couples, it is possible to measure the flow rate \(F = \Delta V/t_{ij}\) with high accuracy, when a differential pressure value \(\Delta P\) is applied across the pipe. The differential pressure \(\Delta P = P_{in} - P_{atm}\) is obtained by filling the experimental setup with pure nitrogen at the pressure \(P_{in}\) with respect to the atmospheric pressure \(P_{atm}\). The pipe containing the liquid is connected by means of a valve \(V_1\) to a gas tank with volume of 5000 \(cm^3\). The gas tank is connected to a nitrogen cylinder by means of a valve \(V_3\). A differential pressure sensor (PS) inserted between the valve \(V_1\) and the gas tank allows...
the pressure $P_{in}$ to be measured with respect to the atmospheric pressure $P_{atm}$. A further, independent valve $V_2$ together with a 0.22 µm filter on top of the pipe allows the liquid substance of interest to be inserted in the pipe itself, which has an overall volume of 10 cm$^3$. The pipe volume is 1/500 of the gas tank volume, so that the pressure value $P_{in}$ and in turn the pressure-drop value $\Delta P$ across the pipe, can be retained constant during flow measurement (maximum relative error on $P_{in}$ between pipe full of liquid and pipe empty is about $2 \times 10^{-3}$). The filter allows avoiding occlusion of the microneedle array due to possible particles with size on the micrometer range in the liquid substance.

Flow rate measurements are carried out using seven, out of ten, consecutive couples of electrodes, which allow six reading of the flow-rate to be obtained for the same $\Delta P$ value. For a given liquid substance, two measurement sessions are performed for each $\Delta P$ value. As an example, Fig. 10 shows the flow-rate readings obtained from each one of the six couples of electrodes for two measurement sessions (circle dot and square dot for the first and second measurement session, respectively) carried out with DIW at different $\Delta P$ values. For a given $\Delta P$ value, the average value of the whole set of twelve measurements (solid lines) is also reported. A maximum percentage deviation (worst case) of 6.6% from the average value is measured for DIW over the whole set of measurements in the whole range of $\Delta P$ values investigated. Similar results are obtained with aqueous solutions of NaCl and D-glucose.

**Evaporation and acceleration losses**

For medical applications, evaluation of the microchip capability of properly storing liquids (for instance, drug to be injected or interstitial fluid to be collected) in the reservoir integrated on the back-side of the silicon die is of chief importance. Losses of liquid through the hollow microneedles can actually occur due to: i) evaporation losses (static losses); ii) acceleration/deceleration losses (dynamic losses).

Evaporation losses are measured at room temperature and atmospheric pressure with the microchip mounted at the bottom of a 10-cm-high graduated pipe filled with deionized water and provided with a 0.22 µm filter on top, with the microneedles pointing outward (see as an example Fig. 9, right). The $\Delta P$ value across the pipe is zero in this case being $P_{in}$ on top of the liquid equal to $P_{atm}$, so that possible losses due to liquid flow throughout the needle array can be ruled out, according to fluidic measurements performed on the microchip at different $\Delta P$ values reported in Fig. 7 that indicate null liquid flow at $\Delta P = 0$. Evaporation losses are measured by monitoring the liquid level in the column as a function of the time. Experimental results, which are reported in Fig. 11, show that evaporation of DIW through the needle array occurs at a rate of about 71 nl min$^{-1}$ (microneedle output open in Fig. 11). Evaporation stops when a lid is applied at the bottom of the pipe in front of the needle array (microneedle output closed in Fig. 11). As soon as the lid is removed (microneedle output open in Fig. 11) evaporation starts again with a rate of the same order of magnitude as before. Slight changes in the evaporation rate in the two phases of the measurement can be explained in terms of small variation of the room temperature and/or humidity level in the measurement environment. The non-measurable liquid losses with the lid placed at the bottom of the pipe confirms that liquid flow throughout the needles can be ruled out, so that measured losses are due to evaporation of liquid throughout the needles. The whole test lasted 456 h (19 days), which means an overall volume variation of 1.56 ml at the evaporation rate of 71 nl min$^{-1}$ and, in turn, a variation of the column height of about 3.10 cm. Evaporation losses through the single microneedle (Fig. 11, right axis) are obtained under the hypothesis that losses through the whole microneedle array is due to the contribution of the 38 000 independent and identical active needles.

Liquid losses occurring as a consequence of unwanted acceleration and/or deceleration, for instance due to accidental fall during transport (shock-test), are monitored by providing the microchip with a sealed container, the latter put on the back-side of the silicon die in order to expand the reservoir up to 0.57 ml (Fig. 12). The reservoir is filled with DIW and the sample is placed on a centrifuge with the needles pointing outward in the direction of the centrifuge force. Acceleration at 80g for 30 s,
with additional ramp-up and ramp-down of 5 s, are used to evaluate liquid losses by comparison of the sample weight before and after each test. This test can be thought of as the worst case of a shock test performed at 80g, since in the shock test the acceleration of 80g suffered by the device under test only takes fractions of seconds. The average liquid losses measured per unit of time were found to be of 1320 nl min \(^{-1}\) with a standard deviation of 346 nl min \(^{-1}\) over a set of 9 measurements.

On the basis of performed tests, it can be concluded that for liquid storage an adhesive cap is sufficient to stop liquid losses through the microneedles.

Conclusions

A silicon microchip for transdermal injection/sampling applications has been designed, fabricated, and characterized. The microchip exploits a high-density array of hollow silicon-dioxide needles with external size of a few micrometers, protruding for one hundred micrometers from the front-side of the silicon die, to inject/draw fluids into/from the skin. The needles are in connection with a number of independent reservoirs integrated in the back-side of the silicon die. Insertion experiments of the microchip in skin-like polymers (agarose hydrogels with concentrations of 2% and 4% wt) has been successfully performed applying a constant force of 200 gf and 500 gf to the chip for 30 s, 60 s, and 120 s, thus demonstrating that such high-density, tiny silicon-dioxide microneedles can successfully withstand penetration without breaking, according to theoretical predictions. Operation of the microchip using different liquids of biomedical interest has been investigated by measuring flow-rate through the needle array as a function of the pressure-drop applied to the chip for injection/drawing purposes, as well as by quantifying evaporation and acceleration losses through the needles for storage purposes. For instance, using an array with 38 000 active needles featuring square-like bore with side of 3.6 \(\mu\)m, i) the flow-rate can be finely controlled from a few ml min \(^{-1}\) up to tens of ml min \(^{-1}\) using differential pressures in the range 10–100 kPa, ii) evaporation losses at atmospheric pressure and room temperature are about 70 nl min \(^{-1}\), and iii) acceleration losses at 80g are about 1300 nl min \(^{-1}\).

In spite of fabrication and operation being demonstrated in this paper for the specific case of a microchip with needle density 1 \(\times\) 10\(^{4}\) cm\(^{-2}\) and multiple reservoirs with volume 0.3 \(\mu\)l each, the fabrication process allows high flexibility to be achieved both in terms of needle and reservoir specifications, so enabling tailoring the microchip to different transdermal applications. In fact, this work represents the first step towards the realization of a fully integrated, minimally invasive, transdermal glucose meter, which envisions the future realization of a new-concept micro-needle-based artificial pancreas.

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References