



Review

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Flexible neural probes: a review of the current advantages, drawbacks, and future demands

Sara PIMENTA^{1,2✉}, João R. FREITAS¹, José H. CORREIA^{1,2}

¹CMEMS-UMinho, University of Minho, Guimarães 4800-058, Portugal

²LABBELS-Associate Laboratory, Braga/Guimarães, Portugal

Abstract: Brain diseases affect millions of people and have a huge social and economic impact. The use of neural probes for studies in animals has been the main approach to increasing knowledge about neural network functioning. Ultimately, neuroscientists are trying to develop new and more effective therapeutic approaches to treating neurological disorders. The implementation of neural probes with multifunctionalities (electrical, optical, and fluidic interactions) has been increasing in the last few years, leading to the creation of devices with high temporal and spatial resolution. Increasing the applicability of, and elements integrated into, neural probes has also led to the necessity to create flexible interfaces, reducing neural tissue damage during probe implantation and increasing the quality of neural acquisition data. In this paper, we review the fabrication, characterization, and validation of several types of flexible neural probes, exploring the main advantages and drawbacks of these devices. Finally, future developments and applications are covered. Overall, this review aims to present the currently available flexible devices and future appropriate avenues for development as possible guidance for future engineered devices.

Key words: Brain knowledge; Flexible interface; Multifunctional probe

1 Introduction

The human brain comprises approximately 86 billion neurons, generating and transmitting information in complex patterns (Luo et al., 2022). Brain diseases, such as epilepsy, amyotrophic lateral sclerosis, dementia, and stroke (Zhao et al., 2022), affect humans' quality of life. Knowledge about the brain and its working mechanisms plays an important role in the potential to cure brain diseases in the future, or at least to reduce their effects, through the development of innovative treatments. Implanted neural probes are important devices used to record or modulate neural activity, and recent decades have been marked by a huge technological development of these tools (Wen et al., 2019; Cointe et al., 2022). Implanted neural probes are routinely used in animal studies and are clinically employed for deep brain stimulation (DBS), which

is a treatment for Parkinson's disease, epilepsy, depression, and obsessive-compulsive disorder (OCD) (Dougherty, 2018; Wen et al., 2019; Chapelle et al., 2021; Cointe et al., 2022; Li et al., 2022; Mohammadi et al., 2022).

Conventionally, implanted neural probes are defined as devices with electrical conductors (electrodes) that come into contact with the aqueous ionic solutions of brain tissue and are able to record the electrical activity of a small population of neurons (local field potential (LFP)) and individual neurons (single unit activity or spikes) (Goncalves et al., 2017; Cointe et al., 2022), or to perform electrical stimulation or inhibition of neural activity (Zhao et al., 2022).

Early implanted electrodes were based on insulated metallic microwires or cone electrodes. Subsequently, the strong development of microelectronics in the 1970s led to the implementation of rigid, patterned, and micromachined neural probes, such as the Michigan probes (Wise et al., 1970) and Utah arrays (Jones et al., 1992), which are the most well-known tools widely used in neuroscience research (Rivnay et al., 2017; Choi et al., 2018; Rodrigues et al., 2021). Despite the great findings and advances achieved with

✉ Sara PIMENTA, sara.pimenta@dei.uminho.pt

Sara PIMENTA, <https://orcid.org/0000-0002-6061-320X>

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rigid neural probes, the nature of these tools leads to gradual device encapsulation and degradation due to the immune response generated by the neural tissue (Rivnay et al., 2017).

The immune response generated by the neural tissue can be minimized using neural probes that mimic the flexibility, softness, and micron-scale movements of the brain (McGlynn et al., 2021). Following this necessity, the fabrication of neural probes after the 2000s involved the use of soft polymeric materials as substrates, e.g., polyimide (Rousche et al., 2001), polydimethylsiloxane (PDMS) (Zhao YW et al., 2018), parylene (Rodger et al., 2008), and SU-8 (Altuna et al., 2013). The main drawback of flexible polymeric neural probes is their challenging implantation, due to the high probability of probe buckling (McGlynn et al., 2021; Freitas et al., 2022). However, there are many strategies to overcome this issue, including coating the probe with absorbable molecules (e.g., polyethyleneglycol (PEG)) or using mechanical carriers to guide the probe to the brain region of interest (Ahmed et al., 2021).

The last two decades have also been marked by an evolution of neural probes in terms of their functions and applicability, with the appearance of probes that integrate not only electrical but also optical elements (Kim et al., 2013; Reddy et al., 2019) (e.g., micro-light-emitting diodes (μ LEDs)), probes with a high density of microelectrodes (Pimenta et al., 2021), or even probes that integrate microchannels for the delivery of chemicals (Wen et al., 2019). Fig. 1 shows an overview of the evolution of neural interfaces, presenting the most relevant marks. Some of the concepts presented in Fig. 1 will be explored in more detail in this paper, specifically the flexible neural probes and the novel interfaces.

Due to the high availability of neural devices, a review of the current devices and future suitable avenues for development is crucial. This paper presents a review of the developed flexible neural probes, covering multiple functions and applications (electrical, optical, and fluidic interactions). Their fabrication, characterization, and *in vivo* validation will be covered to analyze the main advantages and drawbacks of these flexible neural devices. Finally, future developments and applications will be presented.

2 Electrical flexible neural probes

As previously mentioned, a neural interface was conventionally defined as an insulated substrate with electrical contacts to record or stimulate neural activity. Flexible neural probes appeared in the 2000s, mainly to overcome the mechanical mismatch between rigid probes and soft neural tissue. Moreover, flexible neural probes are especially suitable for long-term *in vivo* recordings (chronic neural experiments), even for larger neural probes that can integrate a higher number of recording sites (Pothof et al., 2016; Vomero et al., 2022). As an example, Vomero et al. (2022) reported that larger polyimide flexible neural probes (100 μ m width) can lead to more cellular and molecular changes in the probe/tissue interface, but do not compromise the quality of *in vivo* recordings, at least within 12 weeks, compared with thinner polyimide neural probes (30 μ m width).

Polyimide is one of the most used polymeric substrates for implementing neural probes with electrical interaction. Polyimide can be classified into two main classes—photosensitive and non-photosensitive. Rousche et al. (2001) reported a flexible polyimide-based

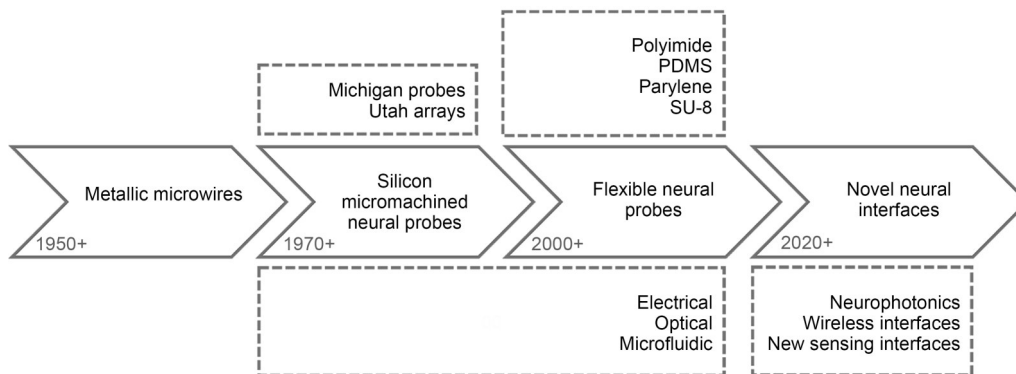


Fig. 1 Overview of the evolution of neural devices. PDMS: polydimethylsiloxane.

intracortical electrode array. The four-shaft device had two microelectrodes per shaft, gold (Au) electrodes sandwiched in a flexible, electrically insulated, and photosensitive polyimide. After device fabrication using standard technologies, Rousche et al. (2001) studied the electrical and mechanical characteristics using impedance measurements and buckling tests. In addition, bioactive capability tests were performed to assess the long-term performance of the polyimide-based array with bioactive species attached. Rousche et al. (2001) confirmed the potential of this kind of interface for extended implant applications in the brain. The recording capability of this interface was also evaluated through its implantation in rats (after pial incisions), and multiunit neural activity recordings were obtained in anesthetized and fully awake animals. Takeuchi et al. (2004) presented a three-dimensional (3D) flexible multichannel neural probe array made of non-photosensitive polyimide. Each probe had three recording pads (Ti (titanium) pads) and it was first patterned on a plain substrate. These probes had a magnetic plate in the backside (electroplating nickel), standing up (forming 3D structures) when a magnetic field was applied. Standard microfabrication technologies were also used for probe implementation. The probe array was characterized in terms of the impedance of the electrode pads and inserted into an anesthetized rat (after peeling off the dura mater), successfully recording spontaneous neural activity from the visual cortex. Xiang et al. (2014) reported an ultra-thin flexible neural probe made of non-photosensitive polyimide with four Au microelectrodes. The fabrication of this ultra-thin size device, using several microfabrication techniques, reduces the immune response of the brain tissue even further. However, a biocompatible and biodegradable maltose layer was coated on the neural probe to facilitate its implantation; this layer was easily dissolved by bodily fluids. Additionally, the Au microelectrodes were coated with a layer of carbon nanotubes (CNTs) to decrease the impedance of the microelectrodes, improving recording capabilities. This probe was characterized with impedance spectroscopy, confirming the reduction of impedance with the CNTs. Moreover, buckling force and maltose dissolution tests were performed, proving the viability of the probe. Finally, the ultra-thin probe was inserted in an anesthetized rat, successfully recording spontaneous neural activity from the hippocampus.

Pimenta et al. (2021) reported a double-layer flexible neural probe with high-density and closely spaced Au electrodes, made of non-photosensitive polyimide. The fabrication of this probe was based on standard microfabrication and cleanroom procedures. The double-layer design of the probe provided a higher number of electrodes (16×2) and additional stiffness to the device, avoiding probe buckling during implantation. The fabrication process was fully validated with chemical composition analysis and metrology of the structural, mechanical, and electrical properties of the device, through energy-dispersive X-ray spectroscopy (EDS) and X-ray photoelectron spectroscopy (XPS), tensile and compression tests, scanning electron microscopy (SEM), and impedance spectroscopy. The applicability of the fabricated neural probe was also accessed through *in vivo* tests in rats, recording spontaneous and evoked neural activity from the cortex with a high signal-to-noise ratio (SNR) and reduced acute trauma. Finally, the insertion footprint was also assessed, *i.e.*, the size of the hole in the rat cortex after probe implantation, which approximately matched the neural probe dimensions. Freitas et al. (2022) presented a flexible neural probe fabricated with a photosensitive and low-temperature cured polyimide. The neural probe had 11 platinum (Pt) electrodes and its fabrication was based on conventional microfabrication techniques, electrodeposition, and annealing/cured processes. The use of a lower curing temperature for the polyimide reduced the thermal oxidation of the metals patterned on the probe substrate. The fabrication process of this device was validated through electrochemical characterization tests, *i.e.*, impedance measurements, which proved the reduction of the impedance of the electrodes after Pt electrodeposition and the future viability of the Pt microelectrodes for neural recordings.

Parylene is also widely used as a substrate for the fabrication of neural probes with electrical contacts. Kuo et al. (2013) reported a parylene-C neural probe with a 3D sheath structure and Pt microelectrodes. The fabrication of this neural probe was based on thermoforming and standard microfabrication processes. The sheath structure could accommodate neurotrophic factors to promote neural tissue ingrowth after probe implantation. Pt microelectrodes were electrochemically characterized with cyclic voltammetry and electrochemical impedance spectroscopy, showing the suitability

of the microelectrodes for neural recordings. Finally, *in vitro* tests of the neural probe implantation in an agarose brain model were performed, using an introducer tool attached to the neural probe with PEG. Castagnola et al. (2015) presented a parylene-C flexible neural probe with poly(3,4-ethylenedioxythiophene) (PEDOT)-coated microelectrodes. The neural probe was fabricated using standard technologies and electropolymerization. The PEDOT microelectrodes were characterized using electrochemical impedance spectroscopy and cyclic voltammetry, proving their viability for neural recordings. Electrophysiological measurements were also performed (*in vivo* in anesthetized rats and *in vitro* brain slices), confirming that PEDOT-coated electrodes enabled the recording of good electrophysiological signals with a very high SNR. Zhao ZG et al. (2018) reported a flexible neural probe based on a parylene tube shank with an 18.2-mm length, which can reach deep brain structures, with Au electrodes. This device was implemented with conventional microfabrication processes and it was characterized mechanically (insertion force tests) and electrochemically (impedance spectroscopy). Finally, the probe was tested in anesthetized rats, allowing the recording of LFP data from the amygdala (one of the deepest brain regions).

SU-8 is also used for the fabrication of electrical flexible neural probes. Altuna et al. (2012) presented an SU-8-based probe with planar Au electrodes for neural recording. The neural probe was implemented using conventional microfabrication processes. The viability of the Au electrodes was confirmed through electrochemical impedance measurements. Finally, the neural probe was implemented in an anesthetized rat, successfully recording action potentials and LFP data from the dorsal hippocampus.

Overall, the previous paragraphs present the most common electrical flexible neural devices based on polymeric substrates (Table 1). Fig. 2 also shows some of the reported electrical flexible neural probes implemented in polyimide (Fig. 2a), parylene (Fig. 2b), and SU-8 (Fig. 2c). Other flexible neural probes have been developed by several researchers, including flexible interfaces with an ultra-high density of electrodes (Chung et al., 2019), nanoelectronic flexible interfaces (Luan et al., 2017; Tchoe et al., 2022), and devices with not only electrical interaction but also optical and microfluidic capabilities; these will be explored in

Sections 3 and 4, respectively. Finally, it is important to state that the main drawback of flexible neural probes is the difficulty of implanting them into the brain. The three main strategies reported in the literature to overcome this problem are: (1) the use of an auxiliary introducer tool (e.g., stainless steel wire) (Rousche et al., 2001; Kuo et al., 2013; Zhao ZG et al., 2018); (2) the coating of the neural probe with an absorbable molecule (e.g., maltose, PEG, saccharose, or hydrogels) to temporally confer more rigidity (Xiang et al., 2014; Weltman et al., 2016; Lecomte et al., 2018; Na et al., 2020); and (3) increasing the thickness of the neural probe (e.g., with a polymeric multilayer) to make the probe body more robust to facilitate its handling and implantation (Altuna et al., 2012; Pimenta et al., 2021).

Finally, responsive polymer-based neural probes are also an interesting engineering approach, which consists of softening neural implants that change their elastic modulus under physiological conditions. These polymers can be chemically activated or temperature-activated, decreasing their Young's modulus after insertion in the living tissue, but keeping it at GPa during its handling and implantation. Nguyen et al. (2014) reported an investigation of the long-term chronic neuroinflammatory response of implanted mechanically-adaptative intracortical microelectrodes. They performed *in vivo* experiments in rats with rigid silicon (Si) implants (for comparison) and poly(vinyl acetate)/tunicate cellulose nanocrystal (tCNC) nanocomposite (NC) implants, which are initially rigid but become compliant after implantation. An immunohistochemistry analysis of the rats' tissues several days and weeks after implantation was performed. Nguyen et al. (2014) concluded that compliant intracortical implants can reduce the neuroinflammatory response in comparison to stiffer systems. Simon et al. (2017) reported, for the first time, the design, development, and validation of an intracortical probe with a tunable elastic modulus (from 2 GPa to 50 MPa), using thiol ene/acrylate thermoset polymer as the probe substrate. The fabrication of the probe was performed with standard microfabrication techniques, obtaining a geometry similar to that of commercial probes (NeuroNexus architecture), with Au microelectrodes that were coated with PEDOT to improve their impedance. Simon et al. (2017) demonstrated the *in vitro* biocompatibility of the probe and its suitability for *in vivo* cortical recordings

Table 1 Electrical flexible neural devices based on polymeric substrates

Reference	Substrate, recording sites	Fabrication technologies	Characterization and/or validation
Rousche et al., 2001	Photosensitive polyimide, Au electrodes	Lithography Electron-beam Thermal evaporation Reactive ion etching Wet etching	Impedance measurements Bucking tests Bioactive capability tests In vivo recordings in rats
Takeuchi et al., 2004	Non-photosensitive polyimide, Ti electrodes	Electroplating Lithography Thermal evaporation Wet etching Reactive ion etching Deep reactive ion etching	Impedance measurements In vivo recordings in rats
Xiang et al., 2014	Non-photosensitive polyimide, Au electrodes coated with CNTs	Lithography Thermal evaporation Electron-beam Reactive ion etching Anodic metal dissolution Electrophoretic deposition	Impedance measurements Buckling tests Maltose dissolution tests In vivo recordings in rats
Pimenta et al., 2021	Non-photosensitive polyimide, closely spaced Au electrodes	Chemical vapor deposition Lithography Sputtering Reactive ion etching Wet etching	Chemical analysis Structural and mechanical tests Impedance measurements In vivo recordings in rats Insertion footprint assessment
Freitas et al., 2022	Photosensitive and low-temperature cured polyimide, Pt electrodes	Lithography Electron-beam Wet etching Electrodeposition	Annealing tests Impedance measurements
Kuo et al., 2013	Parylene-C, Pt electrodes	Lithography Electron-beam Reactive ion etching Thermoforming	Cyclic voltammetry Impedance measurements In vitro implantation tests
Castagnola et al., 2015	Parylene-C, PEDOT-coated electrodes	Chemical vapor deposition Lithography Electron-beam Reactive ion etching Wet etching Electropolymerization	Cyclic voltammetry Impedance measurements In vitro tests in brain slices In vivo recordings in rats
Zhao ZG et al., 2018	Parylene, Au electrodes	Lithography Thermal evaporation Wet etching Deep reactive ion etching	Insertion force tests Impedance measurements In vivo recordings in rats
Altuna et al., 2012	SU-8, Au electrodes	Lithography Sputtering Wet etching Electrochemical etching	Impedance measurements In vivo recordings in rats

Au: gold; Ti: titanium; CNTs: carbon nanotubes; Pt: platinum; PEDOT: poly(3,4-ethylenedioxythiophene).

in rats for more than two months. Shoffstall et al. (2018) performed a study to evaluate the neuroinflammatory response of implanted thiol ene-based shape memory polymers (SMPs). They performed in vivo experiments in rats with bare Si implants (for comparison) and SMPs-coated Si implants. An

immunohistochemistry analysis of the rats' tissues 16 weeks after implantation was performed. Shoffstall et al. (2018) concluded that the neuroinflammatory response (astrocytic scarring) was reduced for the coated implants. Zátonyi et al. (2019) reported a neural probe composed of a custom thiol ene/acrylate thermoset

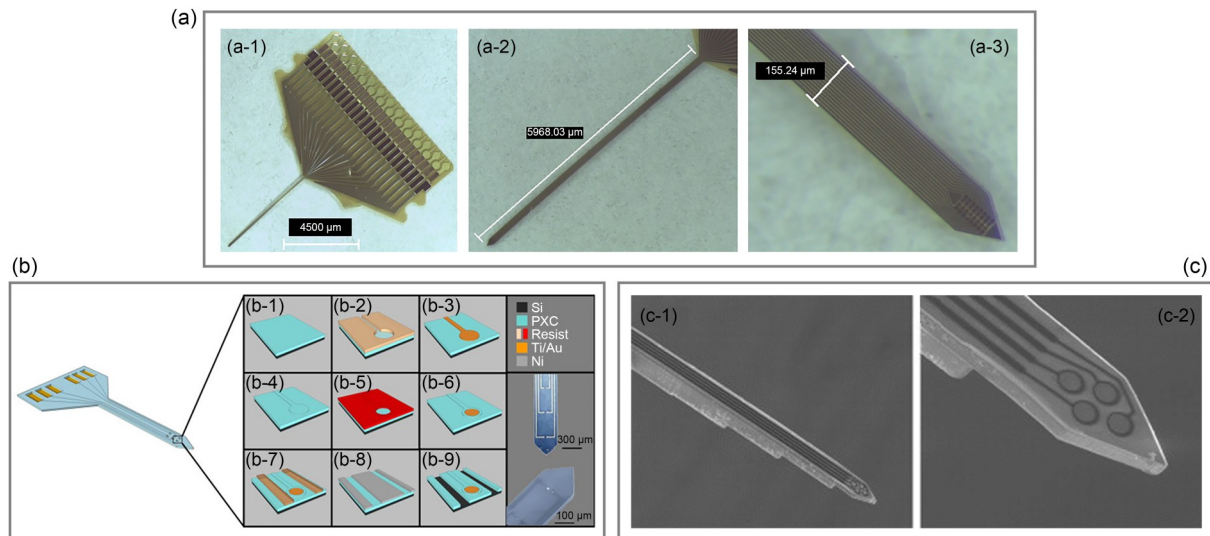


Fig. 2 Illustration of flexible neural probes. (a) polyimide-based, with closely spaced gold (Au) microelectrodes, reported in Pimenta et al. (2021); (b) Parylene-based, with poly(3,4-ethylenedioxythiophene) (PEDOT)-coated microelectrodes, reprinted from Castagnola et al. (2015), Copyright 2015, with permission from Elsevier; (c) SU-8-based, with planar Au microelectrodes, reprinted from Altuna et al. (2012), Copyright 2012, with permission from Elsevier.

polymer with 5-mm length, 200- μm width, and 60- μm thickness. The single shank implant had several 15- μm -diameter Au microelectrodes and its fabrication was based on standard microfabrication processes. After fabrication, the microelectrodes were characterized using electrochemical impedance spectroscopy, and platinization of the microelectrodes was also performed to improve the active surface area. The fabricated neural probe was successfully inserted in animals (acute experiments with rats) without any problems with bending or buckling and without any insertion shuttle, recording single-unit activity from the hippocampus. Zátanyi et al. (2019) also studied the mechanical features of the neural implant with softening polymer samples, performing dynamic mechanical analyses. They concluded that the probe changes its elastic modulus from 2 GPa to 300 MPa when exposed to physiological conditions (body temperature) in a 10-min timescale.

3 Optical flexible neural probes

In 2005, a new concept in neuroscience appeared—optogenetics. This concept combines genetics and optics, where genetically modified neurons are activated or inhibited by light (Goncalves et al., 2017; Freitas et al., 2021). A specific neuron population is genetically modified by the introduction of opsins (light-sensitive

proteins). Those opsins, when activated by light at a very specific wavelength, can stimulate or inhibit neural activity. This capacity for brain activity manipulation generated a variety of neural studies, especially research in brain disorders (Lanzio et al., 2018). Following this new concept of electrophysiology with optogenetics, it was necessary to adapt neuroscience tools and experiments.

Several researchers reported optogenetic studies using optical fibers aligned and glued to neural probes with patterned electrodes (Kim et al., 2015; Lanzio et al., 2018; Pimenta et al., 2021), performing optical stimulation and neural activity recording. This approach does not allow proximity between electrical and optical elements and is not scalable (Lanzio et al., 2018). Other approaches have arisen in recent years, i.e., neural probes integrating electrical and optical elements, which are crucial interfaces for optogenetics studies. These optical elements include μLEDs integrated next to electrodes or waveguides patterned along a neural probe shaft (Fig. 3) (Wu et al., 2013, 2015; Lanzio et al., 2018; Ahmed et al., 2021).

The integration of μLEDs in neural probes has been used for optical stimulation, but mainly on rigid substrates (e.g., sapphire (McAlinden et al., 2015) or Si (Goncalves et al., 2018)), due to the challenging nature of the integration of μLEDs in flexible substrates. Reddy et al. (2019) reported a flexible neural probe

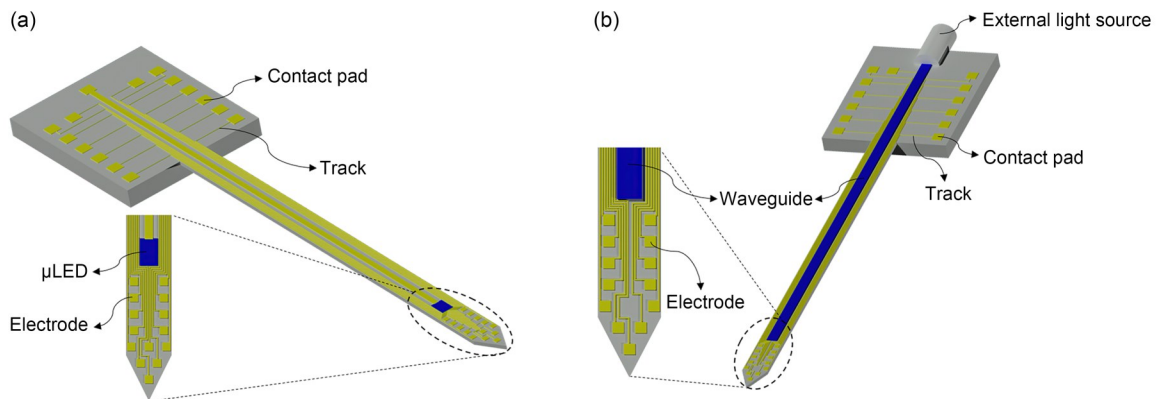


Fig. 3 Artwork of a neural probe with microelectrodes and an integrated micro-light-emitting diode (μ LED) (a) or microelectrodes and a patterned waveguide along its shaft (b).

made of parylene-C, integrating gallium nitride (GaN) μ LEDs and recording electrodes. They demonstrated a complete microfabrication process, comprising the monolithic integration of GaN μ LEDs and Au electrodes on a parylene-C substrate. The fabricated probe was double-sided and its fabrication was based on using a Si wafer with (Al,Ga)N, *n*-GaN, and *p*-GaN epilayers and microfabrication techniques such as photolithography, etching processes, and films deposition. The integration of electrodes and μ LEDs enables simultaneous neural recording and stimulation of the brain, facilitating the study of neural circuits with a higher spatio-temporal resolution. Reddy et al. (2019) performed the optical characterization of the μ LEDs (intensity peak wavelength and output power) and electrochemical characterization of the electrodes (impedance and phase plots). Additionally, they performed thermal simulations to study the effects of local heating in the μ LED on the tissue. Finally, the device was tested with brain slices, indicating that the light from μ LEDs can activate the neurons that express the used opsin.

As previously mentioned, the other possibility for performing optical stimulation is to integrate a waveguide in the neural probe, delivering guided light to neurons. Kampasi et al. (2020) reported the design and microfabrication of a flexible neural probe with a waveguide and recording electrodes. The flexible device was fabricated in polyimide, integrating a polymeric waveguide (polymethyl methacrylate (PMMA)-based material) and Pt/iridium (Ir) electrodes. After fabrication, Kampasi et al. (2020) performed the optical characterization of the waveguide, concerning transmission losses, and the electrical characterization of

the electrodes, accessing their impedance. Thermal simulations were performed to study the temperature rise in the tissue surrounding the device. With these characterization tests, Kampasi et al. (2020) concluded the suitability of the device for long-term chronic brain studies, offering a compact, thermally safe, and flexible solution. Zhou et al. (2022) reported a silk-based neural probe, consisting of a silk protein optical fiber glued to multiple flexible polyimide arrays with Au microelectrodes. After fabrication, using standard microfabrication processes, they demonstrated the capability of the neural implant to perform optical stimulation and electrical recording of neuronal activity in mice. Moreover, behavior studies during optical stimulation in freely moving animals were also successfully performed. Finally, the authors proved the chronic stability and biocompatibility of this neural implant through immunohistochemical analysis.

The integration of a thin-film-based waveguide in a neural probe shaft is also an interesting solution (Wen, 2018). Silicon nitride (SiN) is widely used as a waveguide core material due to its high refractive index (about 2), confining the light inside the waveguide. SiN for waveguides is usually deposited by chemical vapor deposition (CVD)-based processes (plasma-enhanced CVD (PECVD) or low-pressure CVD (LPCVD)). However, for temperature sensitive substrates (such as polymers), it could be more beneficial to deposit SiN thin-films by sputtering, which can vary the final optical and mechanical properties of the SiN films. Some strategies could be used to improve the final properties of SiN-sputtered films, mainly based on the control of several deposition parameters (e.g., sputtering power, gas ratio, and sputtering target)

(Vila et al., 2003; Hegedüs et al., 2022; Tsuchiya et al., 2022). Titanium dioxide (TiO_2) thin films can be also used as a waveguide core material, specifically in visible and near-infrared spectral regions, due to their transparency in that spectral region, long-term chemical stability, high refractive index (>2.2), and low commercial cost (Jurado-González et al., 2023). It is also important to note that the waveguide cladding material could be a lower refractive index material, such as silicon dioxide (SiO_2) or alumina (Al_2O_3), easily deposited by atomic layer deposition (ALD). The use of thin-film ALD for the fabrication of optical devices, specifically for waveguide cladding, can reduce the waveguide surface roughness, improving its performance, i.e., reducing optical losses (Khanna et al., 2014). Moreover, ALD allows the deposition of very thin cladding layers with excellent conformality, reproducibility, and precision (Testa et al., 2010).

The integration of μLEDs or waveguides in a neural interface creates some important issues. One of the most relevant is heat generation, which is especially critical for the μLEDs due to the Joule effect on the interconnect lines that power the μLED and on its surface (McAlinden et al., 2013; Lanzio et al., 2018; Freitas et al., 2021). Waveguides have the advantage of keeping the light source outside the brain, making it possible to change the delivery light's wavelength according to the application (e.g., neural stimulation or inhibition) (Pisanello et al., 2016) or control the optical power for optogenetics activation (Pimenta et al., 2018b). The increase of the neural probe shaft thickness can also be an issue, due to the large size of μLEDs and waveguides (Lanzio et al., 2018), which

can increase the damage to neural tissue after probe implantation, even when using a flexible interface.

Finally, other optical elements can also be considered for integrating the neural probes, such as microlenses on the top of μLEDs . The use of microlenses allows light collimation (Rocha et al., 2014), which can improve the irradiance in the neurons without increasing the μLED power consumption, minimizing the overheating of the brain tissue (Freitas et al., 2021). Freitas et al. (2021) reported the simulation, fabrication, and characterization of a PDMS microlens to be used with a μLED for optogenetics (Fig. 4).

4 Microfluidic flexible neural probes

Some drugs can be used therapeutically to treat brain diseases such as tumors, neurodegenerative diseases (e.g., Alzheimer's), Parkinson's, and epilepsy. However, the standard injection of drugs for the treatment of brain diseases is hampered by the blood–brain barrier (BBB), which is a diffusion barrier essential for the normal function of the brain that maintains brain homeostasis. The majority of biotechnology drugs are large molecules that cannot cross the BBB. One of the strategies to overcome this issue can be the direct infusion of a drug into the brain tissue using an implanted neural probe, a concept named convection-enhanced drug delivery (CED). CED allows the delivery of drugs locally, reducing their effects on healthy tissue and other organs (Fernández et al., 2009; Pardridge, 2012; Lu et al., 2014; Dong, 2018; Ma et al., 2020; Thakor et al., 2020).

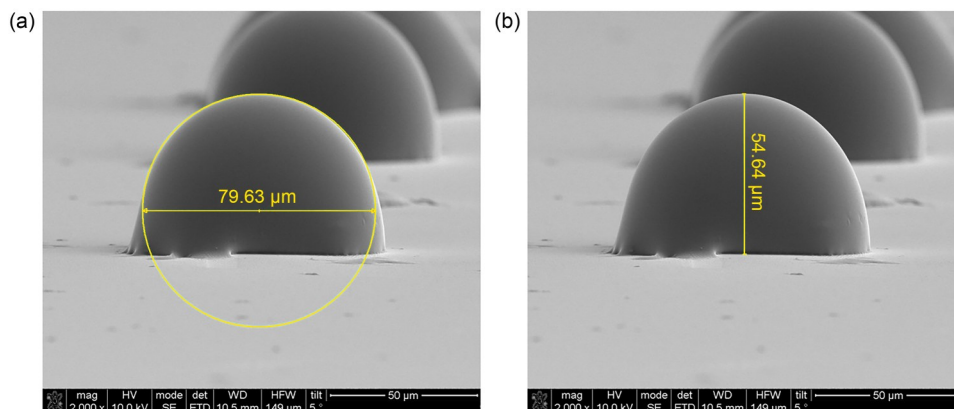


Fig. 4 Scanning electron microscopy (SEM) images of a microfabricated polydimethylsiloxane (PDMS) microlens, showing its diameter (a) and height (b), reprinted from Freitas et al. (2021), Copyright 2021, with permission from Elsevier.

At the beginning of the 2000s, there was a development in Si micromachining-based microfluidic devices to accomplish CED. However, while the rigid neural probes induce more brain tissue damages, polymeric neural probes with microfluidic channels appeared to reduce neural tissue trauma (Fernández et al., 2009). Metz et al. (2004) reported a polyimide neural probe with Pt microelectrodes and microfluidic channels for monitoring electrical activity and delivering drugs. The fabrication process of the device was based on photolithography, thin-film deposition, etching processes, and a lamination method. Metz et al. (2004) also performed the characterization of the microelectrodes and microchannels through impedance spectroscopy measurements and flow experiments, respectively. The microelectrode impedance was suitable for neural recording and stimulation. The microfluidic system allowed a localized delivery of liquids with precise control of their quantity (picoliter range). Fernández et al. (2009) reported an SU-8 microneedle with Pt microelectrodes and a microfluidic channel with multiple outlet ports. The polymeric microneedle was fabricated using photolithography, thin-film deposition, and bonding techniques. The microneedle was characterized *ex vivo* with rat tissues, testing the capability of the needle to deliver drugs at precise localizations, and also *in vivo* with rats, accessing the tissue damage during the needle insertion.

Besides chemical delivery, a microfluidic channel in a flexible neural probe can serve the function of improving the mechanical stiffness of the probe by filling the microchannel with a rigid material. Takeuchi et al. (2005) presented a parylene neural probe integrating a microfluidic channel and an Au microelectrode inside the microchannel. The fabrication process

of the neural probe involved polymer coating, photolithography, thin-film deposition, and etching processes. The microfluidic channel was filled with PEG, which is a molecule that is solid at room temperature and dissolves when in contact with brain tissue. PEG temporarily increased the probe stiffness, avoiding probe buckling during implantation. Neural signals from a rat brain were obtained with the electrode inside the microfluidic channel. More recently, Wen et al. (2019) reported a PDMS neural probe with Pt electrodes and microfluidic channels (Fig. 5). The authors used gallium (Ga) for the probe fabrication, taking advantage of the solid-to-liquid phase change of the metal at body temperature and thus temporarily increasing the probe's stiffness for implantation. The neural probe was fabricated using standard microfabrication processes (photolithography, thin-film deposition, and etching processes) for microelectrode patterning. Additionally, a PDMS thin-film transfer process was employed to pattern the probe body and the microchannels, and liquid Ga was injected into the interconnects and stiffening channels. The fabricated neural probe was characterized *in vitro* with brain phantoms, testing the stiffness and flexibility of the probe, and also *in vivo* with rats, evaluating the electrochemical sensing and drug delivery functions.

Microfluidic neural probes are certainly important tools in neuroscience research, for the reasons presented in this section. However, as with all neural interfaces, microfluidic devices have some limitations that should be considered, such as the necessity for the probe to be aligned parallel with the insertion direction, minimizing the probability of tissue damage and backflow, and the necessity to precisely control the

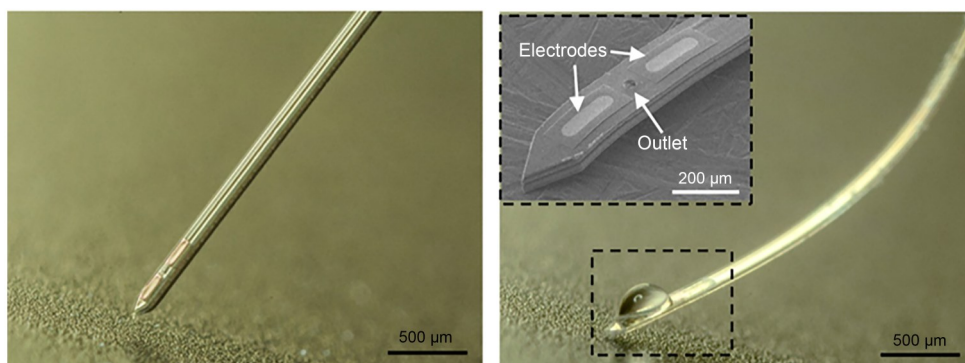


Fig. 5 Polydimethylsiloxane (PDMS) neural probe with electrodes and microfluidic channels, reprinted from Wen et al. (2019), Copyright 2019, with permission from Elsevier.

flow rate, since a high flow rate can cause cell damage. Finally, it is also important to emphasize that microfluidic neural probes need a backside external system with external pumps and tubing connectors (Wen, 2018; Yu et al., 2019; Gupta et al., 2022).

5 New avenues and future directions

Very recently, neural systems capable of performing fluorescence imaging of neural activity have also become a reality (Cecchetto et al., 2021; Sacher et al., 2021). Fluorescence imaging is a great tool for studying brain functionality *in vivo* at several scales, from small neuron networks to the whole brain (Sancataldo et al., 2019). The main goal of neural activity imaging is to generate a record of the physiological state of the brain and its changes over time. Usually, genetically encodable fluorescent indicators are used, such as fluorescent Ca^{2+} indicators of neural activity. These indicators modulate their fluorescence signal in response to a chemical or electrical event in the brain, e.g., neural excitation alters the intracellular levels of Ca^{2+} , increasing the fluorescence emission of the Ca^{2+} indicators (Pimenta et al., 2018a; Kim and Schnitzer, 2022). Sacher et al. (2021) reported the implementation of light sheet fluorescence microscopy (LSFM) in neural studies with animals. To enable tissue illumination and fluorophore excitation, they used an invasive neural probe with a waveguide routing network implanted in the brain, along with gradient index (GRIN) lenses (typical diameters between 0.5 mm and 2.0 mm) for light collection. These GRIN lenses are then coupled to a standalone fluorescence microscope (imaging system) placed outside the animal brain. The development of a flexible neural probe integrating the full illumination and light collection systems (emitters and detectors) for imaging can represent the next generation of devices for performing neural studies with minimal damage to neural tissue and maximal efficiency in collecting optical signal. This is a new paradigm recently referred to in the literature and named integrated neurophotonics (Moreaux et al., 2020). Another crucial aspect of the majority of current neural devices is the existence of external connections with data acquisition hardware and monitoring devices, which hamper behavioral neural studies with freely moving animals (Cho et al., 2021). There is a lack of published

studies performed with awake animals, emphasizing the need to develop wireless neural interfaces (Jendritza et al., 2023). Some possible solutions are the implementation of radiofrequency (RF) data communication systems or even optical wireless communication (OWC) systems with infrared sources on the neural devices (Maiolo et al., 2019; Boulogeorgos et al., 2021). The OWC is a technological trend in biomedical applications, due to abundantly available bandwidth, no interference from other devices, higher achievable data rates, and increased safety (Boulogeorgos et al., 2021). Fig. 6 shows an overview of a possible neural interface for conducting fluorescence imaging of neural activity in freely moving animals, considering some of the main aspects discussed in this paragraph.

Another major concern in the fabrication of flexible neural devices is how to maximize the number of recording sites per shaft. A very recent study (Böhler et al., 2023) reported multilayer polyimide probes with a high density of Pt-recording sites, capable of performing long-term and stable recordings. The fabrication process ultimately comprised five non-photosensitive polyimide layers and seven metallization layers in a probe with a final thickness of 10 μm , following standard microfabrication technologies (photolithography, etching processes, thin-film depositions, etc.).

An additional relevant issue in the fabrication of flexible neural devices is the implementation of biofriendly devices, i.e., devices with improved biocompatibility. Very recently, Wang MH et al. (2022) reported the fabrication of a polyimide neural probe coated with a polypeptide to improve probe biocompatibility. The polypeptide can also serve as a drug carrier due to its special micromorphology. Another recent study performed by Chik et al. (2022) reported a flexible microelectrode array coated with a hydrogel (poly(2-hydroxyethyl methacrylate) (pHEMA)), featuring the reduction of the mechanical mismatch between the probe and the brain tissue.

Hydrogel-based neural interfaces are also a recent trend in neural engineering, reducing the immunochemical rejection of commonly metallic electrodes, especially in long-term implantations. Sheng et al. (2019) described a single fiber of hydrogel (PEG-artificial cerebrospinal fluid (ACSF)) capable of performing optical stimulation and electrical recording in rats. The hydrogel could also serve as a drug carrier.

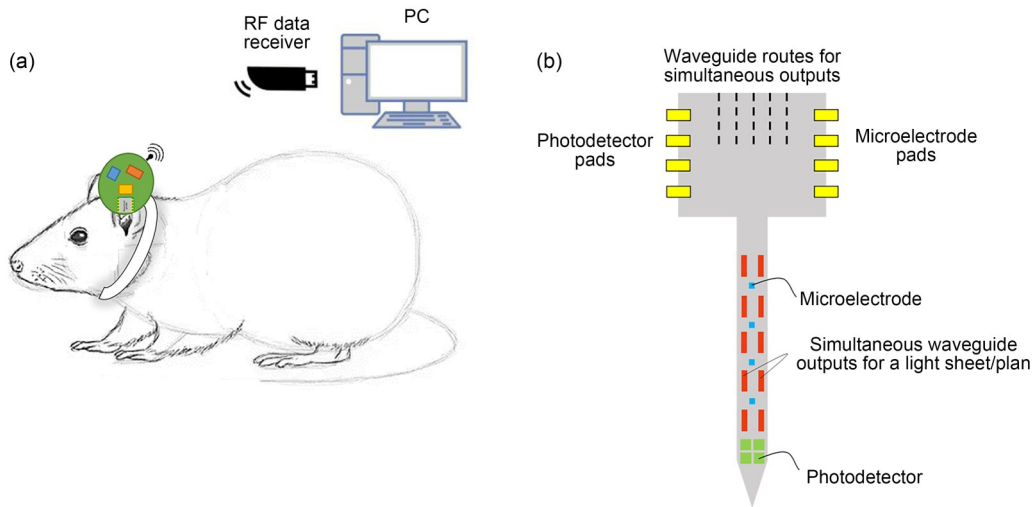


Fig. 6 Artwork of a neurophotonic interface. (a) A reduced dimension and lightweight printed circuit board (PCB) is fixed on the rat’s head with the optical source (that will provide the light for waveguide routes) and all the control, readout, and communication electronics. The neural probe will be coupled to this PCB through wirebonding of pads. (b) Neural probe to be inserted into the rat’s brain. The number of elements (microelectrodes, photodetectors, and simultaneous waveguide outputs) is merely representative. Each waveguide route can lead to two simultaneous waveguide outputs for a specific illumination plane. RF: radiofrequency.

Wang XM et al. (2022) reported the development of a hydrogel-elastomer (polyvinyl alcohol (PVA)-ACSF with PDMS) subdural neural interface capable of recording electrical signals in a cat, with a quality comparable with that of metallic electrodes. The transparent neural interface was also compatible with optical brain imaging.

Finally, the integration of even more functionalities in one device can also be an important requirement to improve the quality and reliability of the experimental data, i. e., creating a device with stimulation and sensing capabilities that is able to record neural signals, stimulate the neurons electrically and optically, and measure, in situ, the temperature, pH, pressure, etc. Flexible electronics have huge potential and can provide several solutions to create a full neural interface able to read a large amount of information relevant to understanding the brain and how to interact with it (feedback system) (Maiolo et al., 2019; Cho et al., 2021).

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Author contributions

Sara PIMENTA was involved in conceptualization, investigation, writing – original draft, writing – review and editing, and visualization. João R. FREITAS was involved in conceptualization, investigation, and writing – review and editing. José H. CORREIA was involved in writing – review and editing and supervision. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Sara PIMENTA, João R. FREITAS, and José H. CORREIA declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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