Commentary

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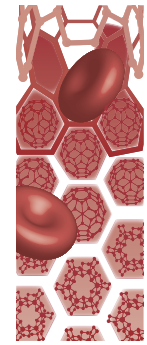
Nanomedicine



Modulation of neuronal firing: what role can nanotechnology play?

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The foremost interest of neuroscience is to find better ways to control neural activity at a single-cell and network level. A successful and punctual neural modulation lies at the basis of any therapeutic strategy for the prevention or recovery of potentially any neurological disorder. Nowadays, clinical neuromodulation at the cortical level finds its benchmark in microelectrodes such as the Utah and Michigan arrays, while deep brain stimulation (DBS) therapies still rely on the macroscale of bulky and invasive multisite electrodes. Notwithstanding, the huge efforts of the scientific community to develop less invasive neuroscientific tools that could preserve a relevant therapeutic efficacy, very few novel strategies stand out as the actual breakthrough.

The potential of nanostructured materials for neuronal stimulation lies in their size and in the variety of mechanisms and interactions that unravel at the nanoscale. Nanotechnological solutions attracted the neuroscientific community looking for higher spatial resolution, specificity and low invasiveness for diagnostics and therapeutics.

Despite the great promise demonstrated by nanotechnology *in vitro*, only a few nanoformulations have reached to date *in vivo* testing or clinical trials, being furthermore mostly intended for imaging and drug delivery. Here, we highlight the state of the art of the few nanotechnological strategies for neural modulation that reached *in vivo* characterizations, discussing the advantages compared with the current stimulation methods that might open a path toward a proficient use of nanotechnology in this field.

Given the increasing number of scientific communications regarding cells and tissues, we propose here an overview of promising nanotechnological tools that already demonstrated a potential for *in vivo* therapeutic strategies. In particular, we focus our attention on active nanostructured devices and colloidal nanostructures.

Nanostructured devices

The majority of *in vitro* studies on neuronal activity employ multielectrode array (MEA) technologies, which allow the spatial mapping of electrophysiological signals over extended networks. Although extremely powerful, these technologies are hampered by the use of extracellular electrodes that do not grant a high signal-to-noise ratio or single cell resolution. On the other hand, patch-clamp techniques provide a higher signal-to-noise ratio and target single cells but are limited to simultaneously recording couples of neurons at best. In order to combine the advantages of the two approaches, some groups have relied on nanotechnologies to create MEAs that are able to adhere tightly to cell membranes and to record even intracellular signals. Spira and colleagues, for example, exploited mushroom-shaped gold electrodes [1] showing how a strategic geometry can create a very narrow cleft between cell membrane and electrode that mimics the typical gigaseal obtained by patch-clamp methods. Another emerging technology employed plasmonic optoporation mediated by gold nanoantennas to achieve high signal-to-noise ratio and intracellular recording [2]. More recently, a novel device with controlled nanoscale rugosity and composed of

4096 nanoelectrodes was engineered for the simultaneous recording of thousands of neurons [3]. Despite the great potential shown for high throughput studies, the application of these nano-electrode devices remains limited to neuronal activity recording *in vitro*.

An interesting approach to mimic patch-clamp capabilities and integrating them with a microelectrode array-based spatially resolved readout, is the use of nano field-effect transistors (nano-FETs). The possibility to tune the amplification of single nano-FETs allows not only for an increased signal-to-noise ratio, but also for a control over single cell activity.

Nano-FETs can be created using a variety of materials and architectures, among which, silicon nanowires represent one of the solutions mostly employed for biomedical applications. In particular, Lieber's group used Si nanowires to measure the extracellular response of spontaneously beating cardiomyocytes [4]. Interestingly, one of the few reports of nanodevices already tested *in vivo* shows the same nanowires successfully used in a 32-microelectrode array configuration and arranged on a flexible injectable mesh architecture that could multiplex local field potential recordings in mice brain [5]. The same group then improved the mesh concept with a macroporous nanoelectronic 3D neural probe integrating flexible FET silicon nanowire technology. The device has been tested *in vivo* for acute local field potential recording with reduced surgical invasiveness compared with standard intracortical recording devices, however still missing the coupling with stimulation [6]. Further improvements brought the number of recording electrodes to 128 [7], while in 2019 a scalable U-shaped nanowire transistor able to measure intracellular activity was proposed and tested *in vitro* [8]. Although this system represents a great solution for recording cellular responses, it still suffers from the fragility of the proposed nanostructures that limits the application mostly to *in vitro*/*ex vivo* conditions. Moreover, this U-shaped transistor solution does not allow cell stimulation.

Alternatively, nanostructured devices with more conventional planar architectures, but employing 2D crystal structures such as graphene, have opened the path to flexible and versatile neural prosthetic devices. Indeed, another solution using FETs has been proposed by Gulmera-Brunet's group exploiting an implantable chip containing 50 µm solution-gated graphene recording electrodes [9]. The unique properties of the 2D nanomaterial proved effective to engineer epi- and intra-cortical foldable electrode arrays. The devices were able to record infra-slow signals, thanks to the persistence of the wide bandwidth FET transconductance and the high electrochemical stability of the graphene gate electrodes directly in contact with the neural tissue. The integration of active components such as transistors in soft bioelectronics opens the path toward the realization of flexible tools for research and clinics, although all the previously shown devices are limited to the recording of neural signals.

The conformability of a device to the diverse anatomical restrictions is indeed crucial in different prosthetics applications. For example, retinal prosthetic devices represent a good benchmark for these emerging nanotechnologies, given the retinal function complexity, the eye's high curvature and the consequent optical aberrations. In this field, ultrathin MoS_2-graphene phototransistors in synergy with neuralinterfacing stimulation electrodes have been proposed as a retinal prosthetic device, demonstrating the possibility to conjugate the required flexibility, highdensity and sensitivity needed in retinal devices [10]. The potential of 2D materials for soft bioelectronics *in vivo* has also been recently shown by a retinal prosthetics prototype combining conjugated polymer thin films and graphene. The promising photosensitive neural interface was exploited to stimulate primary neurons and dystrophic retinal explants, opening the path toward an efficient application of a totally organic and flexible device *in vivo* [11].

Most of the nanotechnologies already proved as feasible tools *in vivo* are incremental improvements of existing stimulation technologies obtained by the addition or incorporation of functional nanomaterials. Guan and colleagues, for example, report on the *in vivo* implantation of a self-powered electronic skin able to store triboelectric energy under body movement and to stimulate mouse hippocampal activity in the CA1 region [12]. $MAPbI_3$ nanocrystals and PDMS, whose activity can be controlled under light-switch in a wireless mode, compose the photosensitive-triboelectric core of such a device. The authors propose the device for a possible application of the perovskite-based electronic skin for *in vivo* characterization of synaptic plasticity, direct neuronal stimulation and ultimately, for improving synaptogenesis and modulate higher brain functions, such as learning and memory.

Another interesting combination of nanotechnological tools with state-of-the-art stimulation technologies employs anatase nanotube films in a photovoltaic device applied to retinal prosthetics in a mouse model of retinal degeneration. The nanostructured film showed a stimulation frequency up to 25 Hz from loose patch-clamp on P23H mice explanted retinas, improving the frequency limit imposed by prosthetics strategies based on continuous films of photoactive material [13]. Similarly, Tang and colleagues successfully integrated a TiO_2 nanowire array with Au nanoparticles in a prototype of retinal prosthetics to enhance the array's photoconversion efficiency in the visible range. The authors show a light-mediated depolarization of retinal ganglion cells by patch-clamp experiments on

explanted retinas from a mouse model of photoreceptor degeneration (*Rd1* mice), and a mild restoration of the light sensitivity through visually evoked potentials in the same animal model after subretinal implantation *in vivo* [14].

Colloidal nanostructures

Most of the nanostructures shown so far enhance the performance of conventional neural devices, while the nanoscale offers the formulation of colloidal dispersions, a major advantage over the macroscale. Colloidal nanomaterials may be treated almost as liquid solutions, earning a fast track to *in vivo* testing thanks to the feasibility of administration by injection. Once injected, the nanostructures can be stimulated by external stimuli like light or magnetic fields, without the need of invasive connections. On the other hand, this kind of strategy faces issues such as loss of targeting due to opsonization and colloidal stability in the biological milieu, recognition and processing by the mononuclear phagocytic system, and long-term accumulation in filtering organs. In the sight of a safe and efficient translation of nanoparticles for neural stimulation into clinical trials, several *in vitro* and *in vivo* studies are devoted to nanostructures biodistribution and fate, blood–brain barrier crossing, and neuronal viability [15,16].

Neuromodulation transduction mechanisms must be addressed also from a safety point of view. In this respect, magnetic fields have well-known tissue interactions and the great advantage of providing a wireless stimulation. The local heating of magnetic nanoparticles by low radiofrequency-alternating magnetic fields has been shown to trigger the opening of thermo-sensitive TRPV1 ion channels, which in turn activate neuronal networks in deep areas of the mouse brain [17]. Another interesting study was based on the expression of chimeric ferritin tethered to TRPV1 channels via a green fluorescent protein nanobody [18]. A similar strategy was exploited to manipulate neuronal activity in the ventromedial hypothalamus of mice, as evaluated by *c-Fos* activation in hypothalamic neurons. The use of this technology was expanded by creating an inhibitory TPRV1, which, if delivered to the hypothalamus of fasted mice, leads to a reduction in blood glucose only in the presence of the magnetic field [19].

Recently, near infrared (NIR)-mediated plasmonic activation of gold nanostructures and its consequent localized heating has been exploited for neural modulation involving TRPV1 channels. A dual system consisting of gold nanorods and TRPV1 channels was introduced in a mouse model of retinal degeneration. NIR stimuli not only enhanced the activity of retinal and cortical neurons but also enabled blind animals to perform a learned light-driven behavior [20]. Remarkably, targeting TRPV1 channels to human retinas, led to the postmortem activation of different cell types by NIR light. Enabling NIR light sensitivity in blind human retinas has important implications as it may result, at least in part, in vision restoration in patients afflicted by neurodegenerative diseases.

The remote photothermal stimulation of neural tissue through plasmonic gold nanorods upon NIR illumination has been shown to trigger temperature-induced capacitance modulation of the cell membranes, that in turn causes depolarization. Carvalho-de-Souza and colleagues have shown that this optocapacitance mechanism is effective on dorsal root ganglia neurons *in vitro* in contact with gold nanorods [21]. Possibly relying on a combination of these photothermal effects, the light-evoked potentials *in vivo* in the sciatic nerve of Sprague-Dawley rats at low irradiance depicted how to extend this stimulation strategy to the peripheral nervous system [22].

Nanostructure-mediated neural stimulation triggered by light covers most of the applications of injectable nanoformulations. Multiphoton absorption is an indirect method that has been proposed to achieve extended tissue penetration, space selectivity and yet visible photostimulation. Indeed, an interesting report shows the possibility to confer NIR sensitivity to mice by the injection of functionalized upconverting nanoparticles (UCNPs) able to bind to the outer segments of the photoreceptors [23]. A similar UCNPs system proposed for DBS was shown to successfully control neuronal functions *in vivo* either by silencing epileptic seizures by inhibition of hippocampal excitatory cells, or by triggering memory recall [24]. Noninvasive activation of brain-injected $NaYF_4$:Yb/Tm@SiO_2 UCNPs by 980-nm NIR generates endogenous blue light emission to stimulate the microbial opsin channel rhodopsin-2 expressed in specific neuronal subpopulations of mouse deep brain areas, resulting in a stimulation of neurotransmitter release and showing the potential of this stimulation strategy for low-invasive therapeutic strategies.

In contrast with inorganic colloidal nanotechnological tools, organic nanotools decline in a variety of approaches that can hardly be covered by this overview, ranging from gene editing to stem cell technologies, from photo-switchable molecules [25] or molecular nanomachines [26] to functional drug release [27]. A few exciting stimulation strategies may be worth mentioning among the vast landscape of organic injectable nanostructures, given their high translational potential.

For instance, photovoltaic conjugated polymers nanoparticles (Poly[3-hexylthiophene-2,5-diyl], P3HT) have been subretinally injected in the eyes of blind rats to restore visual function. P3HT nanoparticles, with a size of approximately 300–400 nm, have achieved a tight contact with the plasma membranes, followed by capacitive

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depolarization of the neuron upon visible light irradiation. The high retina coverage and large interface surface improved the light transduction mechanism and the extent of visual restoration with respect to a continuous film prototype [28].

A different approach employing organic nanotechnological tools for neuronal stimulation exploits nanomaterial cargo capabilities. Li and coworkers developed an approach involving NIR light stimulation of photosensitive hydrogel particles to release locally bioactive molecules, achieving remote control of brain activity without any genetic modification [29]. Glutamate-loaded microgels were injected into the rat cortex, where NIR illumination elicited neuronal firing activity as revealed by microelectrode array recordings.

In a more recent report, encapsulated RuBi-GABA in 100 nm-nanoliposomes were microinjected into the rat brain [30]. An optical fiber was implanted to bring blue light for the activation of the nanoliposome release system, while monitoring both spiking and local field potentials by a customized intracortical MEA. In spite of some limitations due to drug loading and diffusion, this platform effectively induced a prolonged GABA-induced neural modulation *in vivo*, representing a promising strategy for silencing epileptogenic activity.

Caged nanoparticles can also release drugs upon other kinds of physical stimuli. In particular, Airan's group proposed a system composed by polymer nanoparticles able to release propofol upon ultrasound stimulation. They tested these nanoparticles showing that they can reduce seizures in Fischer 344 rats *in vivo* [31], and later proved that the effects they induced are spatially confined to the area under stimulation [32].

Conclusion

This article collects some of the latest reports in which nanotechnology has been demonstrated to be a suitable neuromodulation strategy *in vivo*. Nanotechnological tools for neural modulation stand against the clinical benchmark of DBS electrodes on one side and the state-of-the-art genetic manipulation technologies of optogenetics on the other. Nanodevices mostly relying on standard electrical stimulation represent a reliable way of interfacing with neurons with single-cell resolution and possibly intracellular access, but, apart from few exceptions, the fragility and complexity of their architectures ask for further technological improvement before a feasible application *in vivo*. On the other side, colloidal nanomaterials exhibit the enormous advantages of easy administration, superior spatial resolution and multivalent capabilities, but are still hampered by the incomplete knowledge of their interaction with cells and long-term stability under physiological conditions. Moreover, the immune response to freestanding nanomaterials, that depends upon their size and composition, and their consequent final fate in the body, will probably delay their clinical translatability, although surface biofunctionalization may render nanomaterials fully biocompatible. Nanostructure-enhanced devices exploiting the hybrid combination of well-established technologies with the integration of nanomaterials could prevail in the short term, although mildly improving the landscape of neural modulation performances.

Author contributions

E Colombo, S Di Marco and F Benfenati conceived, structured and wrote the review. All authors participated in writing the review and specifically V Castagnola, ML DiFrancesco and JF Maya-Vetencourt contributed to the colloidal nanostructure section, while G Manfredi and G Lanzani contributed to the nanostructured device section.

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