Mesh electronics: A new paradigm for tissue-like brain probes

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Abstract

Existing implantable neurotechnologies for understanding the brain and treating neurological diseases have intrinsic properties that have limited their capability to achieve chronically-stable brain interfaces with single-neuron spatiotemporal resolution. These limitations reflect what has been dichotomy between the structure and mechanical properties of living brain tissue and non-living neural probes. To bridge the gap between neural and electronic networks, we have introduced the new concept of mesh electronics probes designed with structural and mechanical properties such that the implant begins to 'look and behave' like neural tissue. Syringe-implanted mesh electronics have led to the realization of probes that are neuro-attractive and free of the chronic immune response, as well as capable of stable long-term mapping and modulation of brain activity at the single-neuron level. This review provides a historical overview of a 10-year development of mesh electronics by highlighting the tissue-like design, syringe-assisted delivery, seamless neural tissue integration, and single-neuron level chronic recording stability of mesh electronics. We also offer insights on unique near-term opportunities and future directions for neuroscience and neurology that now are available or expected for mesh electronics neurotechnologies.

Conception of mesh electronics: A historical overview

Limitations of neurotechnologies for probing the brain

Our understanding of the brain has for more than century been advanced by technological breakthroughs [1]. Existing neurotechnologies allow for interrogation and manipulation of the brain activity at different spatiotemporal scales, and are leading to an increasingly better understanding of the brain. Nevertheless, current neurotechnologies remain limited in their capability to cover large spatiotemporal range relevant to understanding the brain; that is, from the spatial scale of individual synapses/neurons with millisecond time resolution to that of neural networks comprising different brain regions evolving over months to years. Functional magnetic resonance imaging can map the longitudinal activity of the entire brain, although is unable to achieve spatiotemporal resolution necessary to follow individual

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neurons underlying observed activity [2]. Alternatively, implanted electrodes can achieve single-neuron level electrophysiology, although with limited chronic recording stability [3,4]. Optical electrophysiology offers high-resolution and relatively large-volume mapping and manipulation of brain activity but has limitations in terms of photon penetration in tissue [5].

The gap between living and non-living systems

Our hypothesis is centered on the observation that brain probes have not been designed to look or behave like the brain tissue, and thus blurring the distinction between the living biological system – the brain – and the non-living electronic system – the probe – will provide new capabilities for addressing fundamental questions in neuroscience and treating neurological/neurodegenerative diseases. Stated in another way, we have worked under the premise that by matching the structural and mechanical properties of the electronic and biological systems, which are traditionally viewed as distinct entities, it should be possible to achieve seamless integration.

The challenges in meeting these constraints are summarized as follows. First, the brain feature sizes scale from tens of nanometers for synapses connecting individual neurons to tens of centimeters for long-range projections integrating distinct brain regions [6]. In comparison, the overall sizes of silicon microelectrode arrays are almost always >4 times larger than a single neuron regardless of channel numbers [7], and microwire-based brain probes become significantly larger than neuron somata with increasing channel numbers, despite subcellular feature size for single-channel carbon electrodes [8,9]. This mismatch in size (Figure 1a, x axis) may contribute to chronic immune response and obscure the natural three-dimensional (3D) connectivity and circuit activity where the probe is implanted [10,11].

Second, brain tissue is very soft with a Young's modulus of 0.1–16 kPa, resulting in a bending stiffness of 10^{-4}-10^{-1} nN·m per unit width of brain slices [12,13]. In striking contrast, brain probes are much more rigid, with bending stiffness values of 10^3~10^5 nN·m (Figure 1a, y axis) [9,14–18]. The large mismatch in bending stiffness results in relative shear motion, glial scar formation and neuron depletion at the probe-brain interfaces, leading to degradation of recording and stimulation capabilities over extended time periods [11,19].

Third, brain tissue comprises organized 3D networks of neurons and non-neuronal cells, such as astrocytes and microglia, which impacts two major points for probe design: (1) The probe structure should not disrupt the 3D connectivity where each neuron is innervated by as many as 10,000 presynaptic endings [6]. (2) The probe should not disrupt the endogenous distribution of cells given their cooperative importance in defining the functional evolution of neural networks [20]. Since solid brain probes exclude a volume of tissue and disrupt the endogenous cell distribution, one should ask whether there are fundamentally new probe concepts that could overcome these limitations.

Seamlessly bridging the brain and electronics: mesh electronics comes of age

Our approach for overcoming limitations of conventional probes and enabling seamless integration of electronics with tissue originated at least decade ago with the convergence of

ideas from two directions focused on interfacing nanoelectronics with biological systems. First and building on our studies of nanowire field-effect transistors (FETs) [21–23], which show readily measured changes in electrical conductivity as the adjacent environment varies, one of us (C.M.L.) suggested implementing these subcellular-size detectors as free-standing 3D neuron-like devices that could interface to live cells via 'artificial synapses' (Figure 1b). Second and recognizing the importance of promoting interpenetration of device arrays with 3D neural networks, C.M.L. proposed macroporous flexible scaffolds to create pathways for cell projections and other cells to 'cross' the device detector plane (Figure 1c) [24]. Together these ideas have driven the realization of mesh electronics with feature sizes similar to neuron somata, mechanical properties akin to brain tissue and mostly free volume that have led to the exceptional properties and opportunities discussed below.

Realization of mesh electronics: Synapse-like nanosensors and macroporous electronic scaffolds

Nanosensors for subcellular resolution recording

A key idea involved in the initial development of mesh electronics was incorporating subcellular-sized sensors to make artificial synapses with neurites and/or enable minimally-invasive intracellular recording [23,25,26]. To this end we first developed nanowire FETs as general biological nanosensors [21,22] and detected propagating action potentials from neurons cultured on arrays of nanowire FETs that formed synapse-like junctions with neurites [23].

Recognizing the limitation of planar electronics for interfacing 3D biological systems also led us to develop the first 3D nanoscale FET cellular probes, in which active FET detectors at the tips of acute-angle kinked nanowires enabled intracellular recording with a point-like detector [27,28]. Compared to other approaches for extracellular and intracellular recording using chip-based organic transistors, nanostraws and nanopillars [18,29–31], the 3D nanoscale FETs allowed localized recording through synapse-like junctions that also could be readily incorporated into macroporous scaffolds as free-standing elements.

Innervated synthetic neural tissue

Initially, our efforts leading to implanted mesh electronics probes for brain science focused on fabrication of active 3D scaffold with addressable nanoelectronic devices followed by cell culture to create innervated tissues. For example, innervated synthetic neural tissue, where rat hippocampal neurons were cultured within a 3D mesh electronics scaffold, led to interpenetrating neural and electronic networks [32]. From a functional perspective, this work further demonstrated recording of highly local field potentials (LFPs) due to postsynaptic signal propagation, owing to the $10^3 – 10^6$ smaller footprint of the integrated nanowire FET sensors versus organic transistors and passive metal electrodes [17,18].

Seamless 3D integration of electronics has been implemented in several types of synthetic tissues (e.g., cardiac and vascular) using nanowire devices capable of detecting chemical signals, mechanical strain and extracellular potentials [32,33], as well as simultaneous electrical stimulation and recording that allows for bidirectional flow of information [34].

For example, innervated 3D synthetic cardiac tissue incorporating both nanowire FET detectors and low-impedance stimulation electrodes within the 3D mesh electronics scaffold affords simultaneous mapping and regulation of action potential propagation in 3D with subcellular spatial resolution and submillisecond temporal resolution [34]. This work has obvious implications for closed-loop cardiac electrophysiology and pacing using implanted mesh electronics, for example, to stimulate tissue foci or the whole ventricle [35] when the ultraflexible mesh electronics is placed conformally on the heart surface.

Mesh electronics for brain science

To move from an *in vitro* scaffold for synthetic tissue to implantable neural probes for in vivo electrophysiology, four key issues must be addressed. First, a minimally-invasive delivery method that affords precise targeting of brain regions needs to be developed for implantation of mesh electronics. Second, the interface between mesh electronics and neural tissue must be characterized to quantify any chronic immune response and probe-tissue interactions. Third, a method to make input/output (I/O) connections is required for in vivo electrophysiology. Last, it is critical to evaluate the single-neuron level chronic recording/ stimulation stability afforded by tissue-mimicking mesh electronics. Below these four key areas are discussed.

Unique delivery of ultra-flexible mesh probes

Mesh electronics probes are designed to have element sizes smaller than soma, bending stiffness values similar to brain tissue, and unit openings >100 times larger than soma. They incorporate arrays of recording/stimulation electrodes with positions defined during fabrication to target specific brain regions, and individually addressed by metal interconnects encapsulated within longitudinal polymer elements, which then terminated at I/O connection pads. The ultra-flexibility of submicron-thick mesh structure is readily apparent in aqueous solution where the 'mesh probes' literally suspend much like colloids (Figure 2a, I) [14,36]. Unlike conventional rigid probes that are directly inserted into the brain at the cost of long-term immune response and chronic recording instability [4,37–39], the ultra-flexibility of mesh electronics opens up a simple solution commonly used in biology for delivery of biomolecules and cells – direct syringe injection through a needle.

Once suspended in aqueous solution, a centimeter-scale mesh electronics probe can be drawn into a syringe needle, and then injected under positive pressure into tissue or solution (Figure 2a, II) [14]. The same flexibility also poses a challenge in precise targeting due to potential crumpling during injection, which could yield ill-defined electrode positions. To solve this challenge, a semi-automated controlled injection method was developed by balancing the injection rate and needle withdrawal using a standard rodent stereotaxic frame (Figure 2a, III), resulting in reproducible fully extended mesh structures in targeted brain regions after implantation [36].

Implanted mesh electronics do not exhibit chronic immune response

Cross-sectional immunohistology studies were used to evaluate the chronic response of neural tissue following mesh implantation [40,41]. Conventional rigid and nonporous

silicon, tungsten and carbon probes typically produce glial scarring and neuron depletion at the probe-tissue interface due to mechanical mismatch between the implanted probes and brain tissue [4,9,11,19,38,39,42,43]. Mesh electronics, by contrast, was designed to "look and behave" like neural tissue both structurally and mechanically to overcome these long-standing issues with conventional probes.

Indeed, immunohistology studies at times up to a year post-implantation [14,40,41,44] demonstrate that the distribution of neuron somata, axons, astrocytes and microglia at the mesh-tissue interface is nearly the same as natural tissue baseline by 4–6 weeks, and maintains this natural distribution to at least a year (Figure 2b&c). Despite the slight elevation of astrocyte and microglia signals at early times, there is no evidence for chronic proliferation of astrocytes and microglia or depletion of neurons; instead, time-dependent penetration of axonal projections and somata into the interior of mesh electronics has been found during the first 12 weeks post-injection. These unprecedented results highlight the seamless neural interface without chronic gliosis and natural distribution of both neurons and non-neuronal cells achieved with mesh electronics, thus raising expectations for stable recording of neural activity critical for advancing fundamental studies and long-term therapeutic implants.

Facile I/O connections for electrophysiology

A critical challenge associated with translating mesh electronics from ex-vivo tissue scaffolds to implantable brain probes involved developing reliable methods for multi-channel I/O connection to standard measurement electronics, since syringe-injection through fine needles makes it topologically impossible to pre-bond I/O pads to connectors. To address this challenge, we have developed computer-controlled conductive ink printing and plug-and-play I/O interfacing methods [36,45]. The plug-and-play I/O interface features an ultra-flexible mesh region with recording/stimulation electrodes to be implanted into the brain tissue, a stem region that routes all interconnect lines, and an I/O region where regular pads are oriented perpendicular to parallel interconnects for plugging into standard zero insertion force (ZIF) interface connectors (Figure 3a, I).

This new design is attractive for general users since it enables "by hand" plug-and-play connection to a ZIF connector after injection (Figure 3a, II). The ZIF connector is mounted on a printed circuit board (PCB) with a standard Omnetics connector, thus resulting in a compact head-stage for acute and chronic multiplexed recording/stimulation studies (Figure 3a, III) [45]. In addition, this compact head-stage can be readily expanded to include multiple ZIF connectors that allow plug-and-play connection and multiplexed recording from multiple mesh probes implanted in different brain regions.

Stable chronic recording and stimulation at the single-neuron level

The above sections set the stage for chronic multiplexed recording and stimulation studies, which have demonstrated single-neuron level brain mapping of the same neurons and local circuits on a year timescale in mice [40]. Several key results from these studies are summarized below. First, 16-channel multiplexed recordings at 2 and 4 months post-injection of mesh electronics yielded stable modulation of LFPs and consistent amplitudes

of single-unit spikes across this two-month period (Figure 3b). Statistical analysis of recording data from multiple mice revealed 85% of channels with identifiable single-unit spikes and on average 2–3 neurons per electrode [46]. In addition, multiplexed data recorded over 6–8 months in different mice showed similar single-unit and LFP stability, despite a gradual increase in single-unit amplitude at early times reflecting the tissue healing process. Moreover, recent studies highlight that further pushing mesh designs towards more neural-network-like and optimizing injection/implantation protocols can reduce and even eliminate the early time amplitude changes (unpublished).

In addition, detailed analyses of recordings revealed stable chronic mapping of multiple neurons and their encompassing neural circuits at the single-neuron level, as evidenced by consistent principal component analysis (PCA, Figure 3c, I), highly similar average spike waveforms, largely unchanged inter-spike interval (ISI) histograms (Figure 3c, II) and stable phase locking to hippocampal theta oscillations across 8 months. The long-term recording stability offers an unprecedented opportunity to carry out longitudinal brain aging studies with single-neuron spatiotemporal resolution. For example, chronic tracking of firing dynamics of the same single neurons showed consistent decline in firing rate (Figure 3c, III) and increase in spike peak-to-trough time for mice aged >48 weeks, providing a new insight into brain aging by revealing the distinct single-neuron changes over extended timescales.

Last, the capabilities of mesh electronics can be readily expanded by incorporating stimulation electrodes to afford simultaneous chronic stimulation and recording at the single-neuron level, where the stimulus-induced artifact in recording can be easily removed owing to its predictable characteristics [47]. Time-dependent studies of post-stimulus spike incidence and latency confirmed stable single-neuron responses to chronic electrical stimulation, highlighting the potential for using multi-functional mesh probes for chronic neuron/circuit modulation and recording studies.

Outlook: mesh electronics for neuroscience and neurology

The unique capabilities of syringe-injectable mesh electronics as tissue-like and seamlessly integrating brain probes suggest a number of exciting directions. Below we highlight three general areas from the perspectives of neuroscience opportunities, neurotechnology development and neurological applications (Figure 4).

Neuroscience opportunities

The unique single-neuron level, long-term recording and stimulation capability of mesh electronics could provide previously unavailable data crucial for understanding many important brain functions and cognitive processes that span orders of magnitude in their relevant time and length scales. For example, conventional low-resolution longitudinal studies [48] and higher-resolution cross-sectional studies [49] are incapable of studying brain aging, cognitive learning and memory and reward circuitry evolution [50,51] by tracking underlying electrophysiological changes at the individual neuron level over months to years in multiple interconnected brain regions. The long-term stability of mesh electronics now makes possible studies of brain circuit evolution over these heretofore missing

spatiotemporal scales, and thus could provide single-neuron/neural circuit level insight into the neurological basis of these important brain functions and cognitive processes.

Neurotechnology development

There is great opportunity for further development of mesh electronics paradigm. For example, owing to the active detector areas that are much smaller than conventional passive electrodes, nanowire FETs are potential candidates for incorporation into mesh electronics to provide highly-localized detection of both extracellular and intracellular field/action potentials in vivo [26,52–54]. Additionally, the ultra-flexibility of mesh electronics and the natural cell distribution post-implantation suggest that functionalization of recording/ stimulation devices with targeting molecules for in vivo neuron-subtype-specific electrophysiology. Moreover, mesh electronics provides a platform for incorporating polymer optical waveguides as a chronically-stable deep-tissue light source for optogenetics, eliminating degradation of the fiber/optrode performance over time due to chronic gliosis that is usually observed for existing rigid optogenetic probes [55].

Neurological applications

Last, we believe mesh electronics offers important opportunities for neurology and clinical translation. First, minimally-invasive syringe injection allows for delivery of mesh probes into virtually any soft tissue in vivo, including the retina, spinal cord and neuromuscular junctions, resulting in injectable neuroprostheses to restore vision and motor functions in models of retinal and muscular dystrophy [42,56]. Second, the chronically-stable and seamless integration afforded by mesh electronics suggests an ideal platform and even a lifespan implant for long-term deep-brain stimulation (DBS) in Parkinsonian patients without chronic gliosis and brain-machine interfaces (BMIs) with single-unit activity based decoding for neuroprosthetic control [57,58]. Third, by understanding and manipulating the extracellular matrix-like properties of mesh electronics to favor migration and development of neural progenitor cells [59], while simultaneously monitoring/modulating neural activity, we envision mesh electronics to serve as an active therapeutic for repairing injured brain regions.

Conclusions

Our goal to bridge the gap between the structure and mechanical properties of neural and electronic networks a decade ago has now led to the realization of mesh electronics that 'look' and 'behave' like neural tissue, evidenced by the lack of chronic immune response, seamless 3D integration with neural tissue, and unprecedented stable long-term multiplexed mapping and modulation of local neural circuits at the single-neuron level. Together, these advances open up exciting opportunities for studies in neuroscience, neurology and further development of the mesh electronics paradigm. Finally, we quote from '*Imagined Worlds*' authored by theoretical physicist and mathematician Freeman Dyson [60]: "*New directions in science are launched by new tools much more often than by new concepts.*" Given the unique advantages offered by mesh electronics as discussed in this review, we are excited to be equipped with a new and general tool that will launch new directions and discoveries at the research frontiers of neuroscience and neurology.

Acknowledgments

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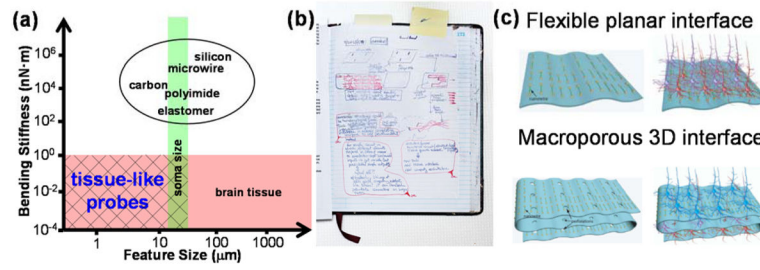60. Dyson, FJ. Imagined worlds. Cambridge, Mass: Harvard University Press; 1997.

Figure 1.
Challenges of state-of-the-art neural probes and original conception of mesh electronics. **(a)** Comparison of feature size and bending stiffness between existing neural probes [7–9,12,15–17] and a 20~100 μm thick slice of brain tissue. Ideal brain probes should have critical feature sizes and bending stiffness values similar to or smaller than those of the brain tissue to afford 'tissue-like probes'. **(b)** Original page from Lieber's notebook in 2008 showing key idea leading to free-standing 3D neuron-like nanoelectronic devices to interface with neurons in a biological manner. **(c)** Schematic drawings presented in 2007/2008 talks and NIH proposal illustrating the key concept leading to mesh electronics with porosity that enables interpenetration and integration of neural networks with electronics structure [24].
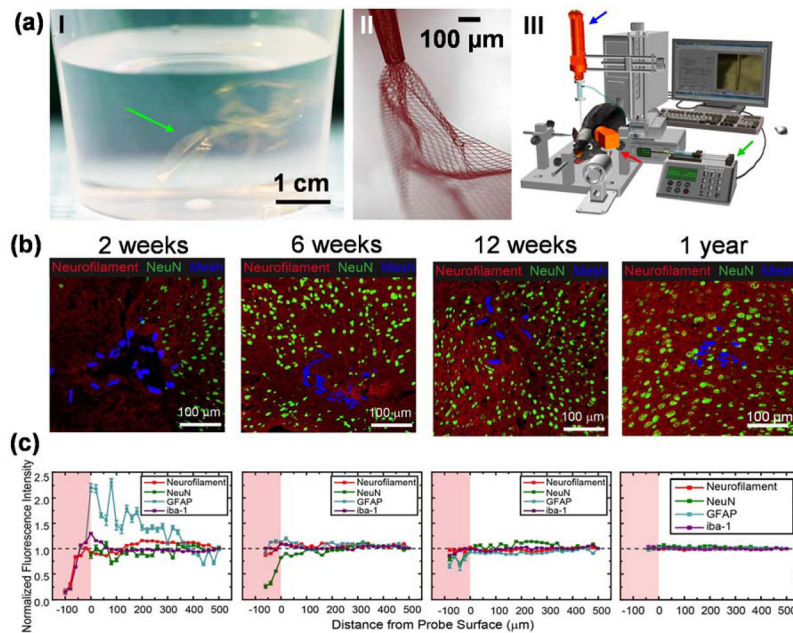
Figure 2.

Syringe delivery of mesh electronics into the brain to yield neuron interpenetration without a chronic immune response. **(a)** Unique structural and mechanical properties of mesh electronics allow for syringe delivery into the brain, highlighting a photograph of multiple mesh electronics probes (green arrow) floating in an aqueous saline solution similar to colloidal particles (I), a bright-field microscope image showing partially ejected mesh electronics with significant expansion in solution (II), and a schematic of controlled stereotaxic injection (III) that allows precisely targeted delivery of mesh electronics using a motorized translational stage for controlling needle withdrawal (blue arrow), a syringe pump for controlling the injection rate (green arrow), and a camera for visualizing the mesh during injection (red arrow) [14,36]. **(b)** Time-dependent immunohistochemical staining images of horizontal brain slices at 2 weeks (hippocampus), 6 weeks (cortex), 12 weeks (cortex) and 1 year (cortex) post injection. In all images of panel (b), red, green and blue colors correspond to neuron axons (Neurofilament antibody), neuron nuclei (NeuN antibody) and mesh elements. **(c)** Normalized fluorescence intensities plotted versus distance from the mesh/ brain tissue interface at different time points; the intensities were normalized versus background far from the probe (black dashed horizontal lines). The pink shaded regions indicate the interior of mesh electronics [40].
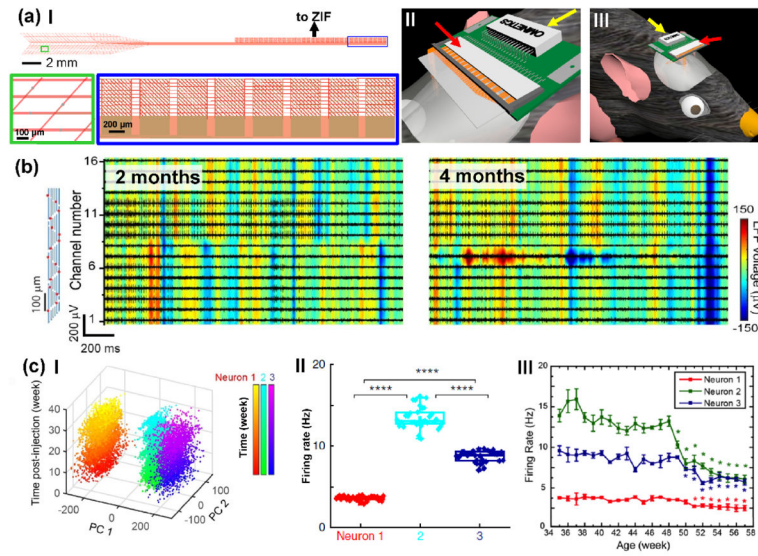
Figure 3.
Electrical I/O connection and long-term stable recording at the single-neuron level using mesh electronics. **(a)** Quantitative and scalable high throughput I/O connection by a plug-and-play interface: structural design of the plug-and-play mesh electronics (I), insertion of I/O pads of plug-and-play mesh electronics into a ZIF connector (II), and compact headstage comprising mesh electronics inserted into the ZIF connector (red arrows) on a PCB that provides an interface to a standard Omnetics connector (yellow arrows) for recording (III) [45]. **(b)** 16-channel multiplexed recording of LFP (background heat map) and single-unit firing (foreground black traces) from the same mouse brain at 2 and 4 months post injection. The relative positions of all 16 recording electrodes are marked by red dots in the schematic (leftmost panel), and span the somatosensory cortex to hippocampus. **(c)** Chronic tracking of same individual neurons by time-dependent PCA (I) and firing rate analysis (II) that allows for study of brain aging on the single-neuron level by tracking firing rate evolution of the same three individual neurons from 35 to 57 weeks of age (III) [40].
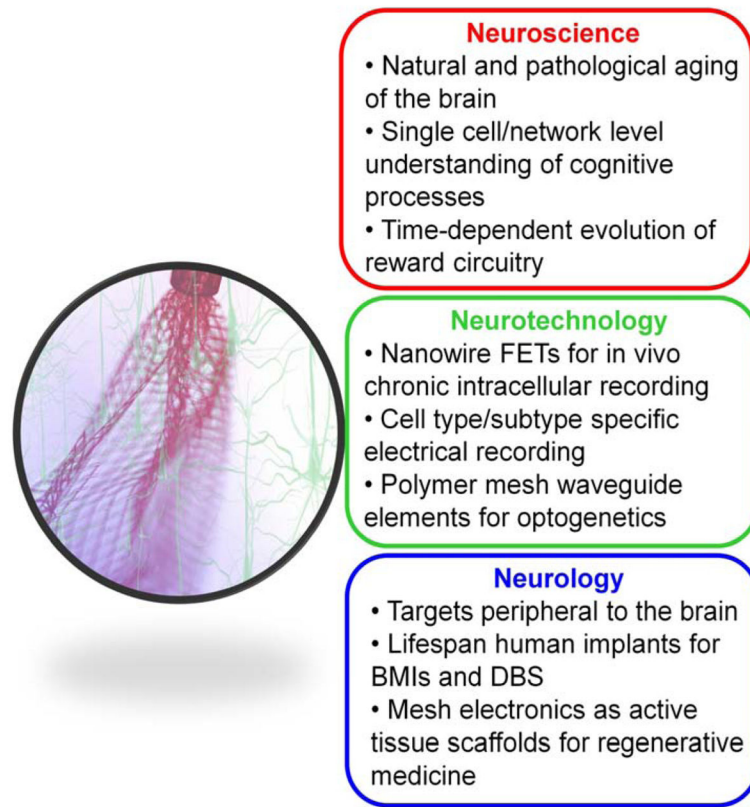
Figure 4.
Outlook and three basic areas of opportunity for mesh electronics neural probes, including neuroscience opportunities, neurotechnology developments, and neurology applications.