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Glial Interfaces: Advanced Materials and Devices to Uncover the Role of Astroglial Cells in Brain Function and Dysfunction

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Research over the past four decades has highlighted the importance of certain brain cells, called glial cells, and has moved the neurocentric vision of structure, function, and pathology of the nervous system toward a more holistic perspective. In this view, the demand for technologies that are able to target and both selectively monitor and control glial cells is emerging as a challenge across neuroscience, engineering, chemistry, and material science. Frequently neglected or marginally considered as a barrier to be overcome between neural implants and neuronal targets, glial cells, and in particular astrocytes, are increasingly considered as active players in determining the outcomes of device implantation. This review provides a concise overview not only of the previously established but also of the emerging physiological and pathological roles of astrocytes. It also critically discusses the most recent advances in biomaterial interfaces and devices that interact with glial cells and thus have enabled scientists to reach unprecedented insights into the role of astroglial cells in brain function and dysfunction. This work proposes glial interfaces and glial engineering as multidisciplinary fields that have the potential to enable significant advancement of knowledge surrounding cognitive function and acute and chronic neuropathologies.

1. Introduction

The classical neurocentric view of brain function states that only neurons are responsible for information processing in the brain by generation and propagation of action potentials, electrical signals that propagate throughout the neuronal network through chemical synapses. However, the human brain includes several types of nonexcitable, glial cells, that are incapable of firing action potentials.[1–4] Glial cells were once considered to only provide trophic and mechanical support to the neuronal network. Over the past four decades though, the relevance of glia has been proven in vitro and in vivo, shifting the neurocentric vision of the structure, function, and pathology of the nervous system to a more holistic perspective. In particular, it is now evident that the role of glia in brain function occurs at a multidimensional spatiotemporal scales.[1,2] Among glial cells (**Figure 1**), astrocytes are tightly linked to neurons and blood vessels, forming the so-called neuron-glia-vascular unit.[5] These types of interactions suggest that astrocyte physiology is crucial for brain function at the synaptic, cell network, and organ scales (**Figure 2**).[2,3] Astrocytes have the well-defined and critical function of regulating the concentration of ions and neurotransmitters, extracellular space volume, and cerebral blood flow. They also actively communicate with neurons and modulate synaptic functions, possibly contributing to information processing that accounts for cognitive abilities.[2–4] Neurological disorders are invariably associated with astrocyte dysregulation, raising the possibility of causal links, especially in cerebral ischemia, glioma, epilepsy, depression, or chronic pain.[6–9] In this view, there is a demand for technologies that are able to selectively monitor and control glia, and meeting this demand is emerging as a challenge across Neuroscience, Engineering, and Materials Science. The complex signaling dynamics underpinning astrocyte functions remain unclear as most of the technologies and tools used to probe and sense astrocytes are derived from those developed to study neurons. Although cutting-edge tools have been developed to study neurons,

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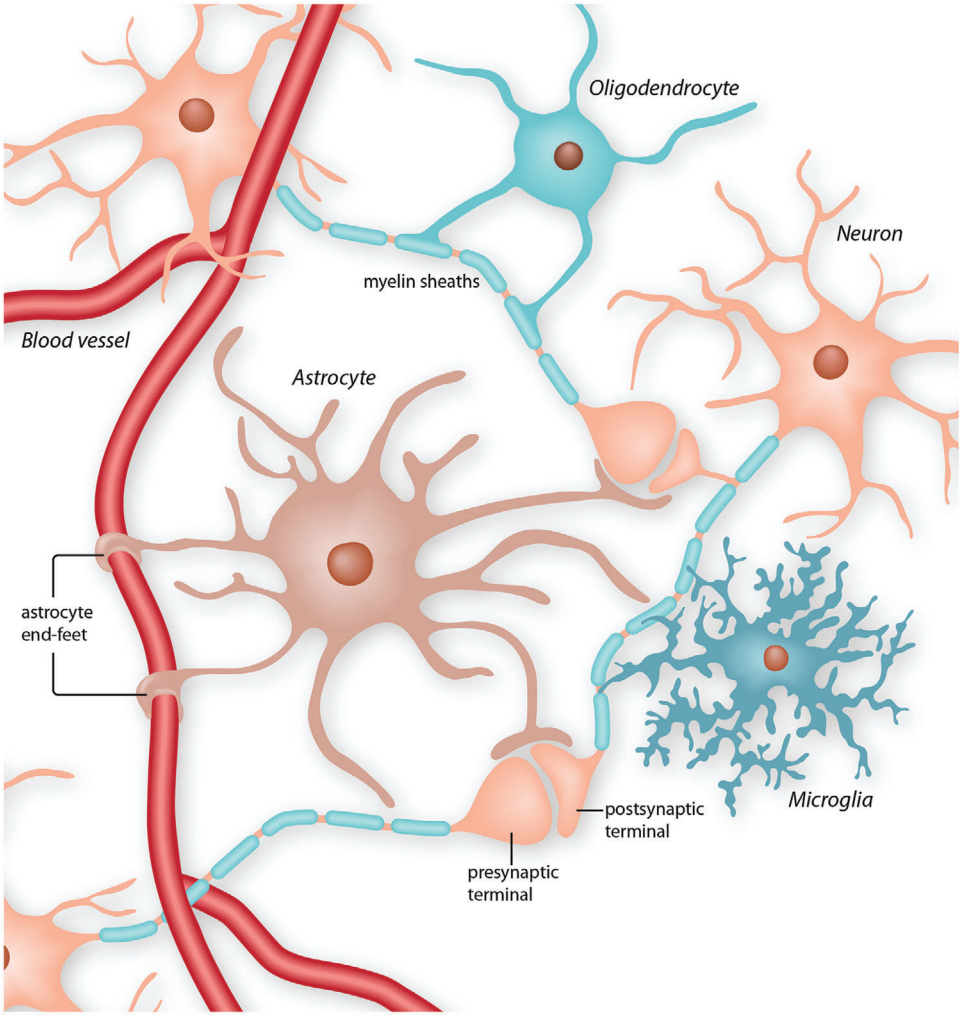

Figure 1. Different types of glia cells in the brain. Schematic draw representing glial cells in the brain and their interaction with neurons and blood vessels. Astrocytes endfeet wrap around synapses and surrounding blood vessels, tightly control the homeostasis of the central nervous system (CNS) and modulating synaptic function. Oligodendrocytes are the cells form the myelin sheaths around axons. Microglia cells represent the CNS immune cells.

they are electrogenic cells and are fundamentally different to astrocytes. Much emerging literature is therefore being devoted to tools specifically developed to study glial cell function.

Major advances in the field of neuro-regenerative medicine will be the successful design and development of biomaterial interfaces and devices that enhance neural tissue regeneration, allow for modulation, and promote functional recovery after brain injury. To accomplish these advances, it is critical to characterize the mechanisms that are responsible for the inflammation caused by brain implants or for the performance failure of implanted devices.[10,11] Importantly, recent reviews have focused on the relevance of studying microglia, oligodendrocytes, and NG2 glia in neuroregenerative medicine.[10,11]

This work aims to fill a gap of knowledge by reviewing the relevance of targeting astrocyte structure and function in biomaterial science and engineering. To this end, this review will provide an overview of the present knowledge of the role of membrane proteins and dynamics of astrocytes in brain physiology and pathology. Then, it will report on biomaterial interfaces, implants, devices, tools, and methods aiming to probe and monitor the structural and functional properties of healthy and pathological astrocytes. The review will critically examine the advantages and pitfalls of the proposed approaches and will provide the roadmap needed to generate devices that i) enable an increased the understanding of the mechanisms underlying the structure and function of astrocytes and ii) can provide unprecedented insights into the multidimensional, spatiotemporal nature of neuron-glial interactions in healthy and/or diseased brains.

1.1. Glial Cells in the Brain

Glial cells include macroglial and microglial cells. Microglial cells orchestrate the immune and inflammatory responses to various brain insults.[7] Since neuroinflammation is a common feature of all brain injuries and diseases, the biological and

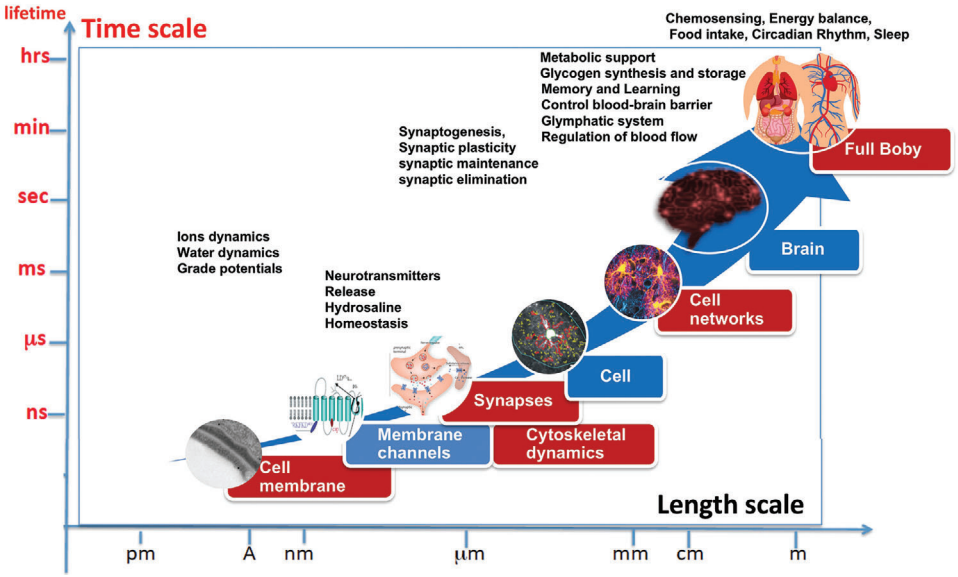

Figure 2. Schematic representation of astrocytic function at multiple biological scales (termed "multiscale") and the implication in brain physiology. The figure correlates neuro-glial dynamic events with the spatiotemporal range during which they occur. Astrocytes contribute to the regulation of synaptic transmission by hydro-saline and neurotransmitter homeostasis through ion dynamics and through neurotransmitter uptake/release by their processes that wrap around synapses, which occur in the ms to seconds range. At the cellular level, they participate in synaptic plasticity, pruning, and homeostasis. The neuronal network activity is modulated by metabolic support, glycogen synthesis, and storage and by local and whole brain regulation of the blood flow. Astrocytes control the blood–brain barrier and liquid flow through the glymphatic system. Over the lifetime, astrocytes have roles in cognitive function, such as memory and learning, and in systemic homeostasis, such as chemosensing, food intake, circadian rhythm, and sleep (insets). Insets named "cell membrane" is adapted with permission.[37] Copyright 2007, Elsevier. Inset named "cell" is adapted with permission.[38] Copyright 2015, Springer Nature. Insets named "cell networks" and "brain" is adapted with permission.[39] Copyright 2003, Cell press.

physiological properties of microglial cells are increasingly attractive for fundamental neuroscience investigations aimed at uncovering the mechanisms behind neurological conditions and diseases. Accordingly, several *in vitro* and *ex vivo* models of microglial cell cultures have been characterized, and their respective advantages and limitations have been recently reviewed.[12] Additionally, transcriptomic, immunohistochemistry, and Fluorescence-activated Cell Sorting (FACS) analyses performed on mice and human ex vivo samples have recently identified the P2ry12 gene and related purinergic signaling[13,14] as unique molecular signatures of human microglia. Microglia can also respond to specific growth factors or cytokines, particularly interferon gamma (IFN-$_\gamma$) and lipopolysaccharide (LPS). These molecules induce microglia to transition their morphology from ramified to amoeboid (blastic) and also promote the structural and functional alterations that characterize "activated" microglia. Moreover, both *in vitro* and *in vivo* studies have confirmed that TGF-β1 is a major differentiation factor for microglia. Activated microglia display phagocytic capabilities and can secrete different types of cytokines and chemokines that are critical to neuroimmune response signaling. Given the crucial role that microglia play in neuroinflammation, the biological properties of microglia have been increasingly considered as targets for improving the compatibility and performance of biomaterials-based, implantable electronic devices, scaffolds, or nanoparticles for drug delivery approaches in the central nervous system.[15] An overview of the possibilities offered by biomaterials-based approaches that target and modulate neuroinflammation has been recently published.[15] On the contrary, the huge potential of therapeutic approaches that target microglial cell biology has received much less attention. In this context, it has been recently highlighted that microglial-mediated neuroimmune response is particularly relevant for chronic diseases, such as neuropathic pain. While it is known that activated LPS receptors—like toll-like receptor 4 (TLR4)—promote the inflammatory microglial response (especially in neuropathic pain), it has been suggested that activation of TLR4, in particular, is majorly implicated in reduced opioid analgesia and in the development of tolerance to and of dependence on opioids.[16] Thus, activation or inhibition of TLR4 by advanced bioelectronic- and/or biomaterials-based devices might represent an innovative approach to counteract neuropathic pain.

It has become evident that quiescent or neutral microglial cells are broadly present in the healthy brain, where they contribute to the maintenance and recovery of homeostasis.[12] Studies *in vivo* have confirmed the ability of microglia to tightly control the structure of the synapse through pruning of unsuitable synaptic contacts and by controlling the neuropil structure and composition.[7] Despite this evidence, the role of microglia in physiological brain function is still largely unclear, mainly due to a lack of appropriate models for studying microglial cell biology. In this respect, recent advances in biomaterials-based surfaces aimed at reproducing a reliable in vitro microglia model are discussed in this review.[17]

Oligodendrocytes are the macroglial cells that synthesize saturated lipids and proteins that form the myelin sheath that surrounds axons and allows for fast saltatory conduction of the action potential along the neuronal axon.[18] During development, myelin formation can be regulated by interaction with other glial

cells, unbalanced concentration of potassium ions, Voltage-gated Na+ blockers and contact with neurons through polysialic acid neural cell adhesion molecule (PSA-NCAM). In addition to their key role in facilitating the propagation of neuronal signals, oligodendrocytes also contribute to homeostatic control of ion concentrations as well as provide trophic and metabolic support. Oligodendrocytes are functionally coupled to each other and to astrocytes through connecting structures called gap junctions (GJs).[18,19] Connexins are the hemichannel membrane proteins that form gap junctions. Connexins are large diameter intercellular pores[20–22] that allow oligodendrocyte-to-oligodendrocyte as well as astrocyte-to-astrocyte diffusion of metabolites, second messengers and intercellular signaling over long-distances (hundreds of micrometers). They also enable the formation of a functional network that is potentially capable of integrating oligodendrocytes as well as astrocytes into the glial syncytia. The panglia hypothesis—stating that astrocytes, oligodendrocytes, and neurons are interconnected by gap-junctional coupling—has been proven by in vivo studies.[23] In this view, the "panglial" network was proposed as an alternative target that would allow for the modulation of neuronal function. Moreover, given their importance in myelin formation and regeneration, oligodendrocytes themselves have become a target of increasing interest for tissue engineering applications aimed at neuroregeneration.[12]

A third type of macroglial cells, called Nerve/glial antigen 2 (NG2) glia and also referred to as oligodendrocytes progenitor cells (OPCs), has been described in the human brain.[24,25] The properties of these cells are peculiar: they are considered a precursor of oligodendrocyte cells in the white matter and remain proliferative in the adult brain. However, NG2 exhibit some functional similarities to neurons, such as the abundant expression of voltage gated sodium Na^+ and Ca^{2+} channels as well as the glutamate receptor alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and GABAA receptors on their plasma membrane.[25] It is now well established that there is cross-talk between the NG2-glia and nearby environmental cells: TGF-β and Bone Morphogenetic Protein (BMP) growth factors, released by epithelial and ependimal cells, can drive NG2 glia migration during development of the Central Nervous System (CNS). In addition, NG2 can interact with neurons through direct excitatory and inhibitory synapses.[26] If the role of NG2 glia in the neural tissue development is becoming more well defined, the physiological relevance of NG2 glia in the adult brain is still unclear. The neuroregenerative potential and self-renewal abilities of NG2 glial cells have been proven in the rodent brain after injury. In particular, after ischemia, the structural and functional properties of NG2 glia can be driven by expression of transcription factors, such as Notch 1, and other growth factors. The permeability of potassium channels expressed by NG2 glia is also altered by ischemic damage, an effect that has been causally linked to the increase of the proliferation of NG2 observed after ischemia.[27] In light of their multipotency, NG2's reprogramming abilities could potentially be exploited in therapeutic approaches for certain neural disorders and diseases.[28] In particular, NG2 glia can transition to myelin-forming oligodendrocytes, or in some cases to neurons, or even more rarely to astrocytes, which might drive a faster and clinically better functional outcome for the injured brain tissue. Because of this evidence, NG2 glia is becoming an attractive target in biomaterials science and engineering.[29] In particular, driving OPCs migration to and their differentiation at the lesion site using a combined drug delivery and scaffolding approach might be a promising strategy for neuroregeneration. In addition, the electrical permeability of NG2 glia can be exploited to trigger or tune their proliferation. In line with these hypotheses, recent work has demonstrated the ability of direct current electric fields to drive NG2 migration in vitro through a mechanism involving the integrin protein $\beta 1$.[30] These findings support the unexplored potential for advanced materials and devices targeting NG2 glia as a potential therapy in neurology.

Astrocytes have long been considered the majority glial cell type in the human CNS. Because of the limited availability of a universal glial marker, the precise ratio of the different glial cells in the human CNS is still under debate. According to morphological studies, astrocytes may account for up to 40% of all glial cells, oligodendrocytes for up to 75%, and microglia and NG2 glia for up to 5–10%. Regional differences are also known to exist and might explain the discrepancies observed among different authors.[1,3] Studies based on phylogenetic evidence highlight anatomic and possibly numerical differences[3] between astrocytes in the human brain compared to those in other mammals.[3] In humans and in higher primates, astrocytes display a distinct complexity, size, and specific cell subtypes. Human cortical protoplasmic and fibrous astrocytes are indeed larger (≈threefold) in diameter and structurally more complex than those of rodents.[3,4] Moreover, subtypes of astrocytes, which are not represented in rodents, have been identified in the human cortex, demonstrating evidence of an increased astroglia heterogeneity among species. The relative number of astrocytes rises impressively with phylogenetic and brain complexity (**Figure 3**). The glia-to-neuron ratio increases from ≈0.05/0.1 in invertebrates to higher values in mammals with dramatically increase in the human cortex, where there are 1.4–2 astrocytes for every neuron.[1,3,4] Moreover, the volume occupied by astrocytes in the human brain is almost 16 times higher than the volume occupied in the rodent brain.[1,3,4] Based on this evidence, intriguing hypotheses are being postulated about the roles of increased astrocytic complexity and numerical expansion of astrocytes during evolution. Is either or are both correlated with increased functionality and possibly cognition of the adult human brain? Whether the data reflect a higher demand for homeostatic clearance and energy support due to increased synaptic transmission[1] or a more complex involvement of astrocytes in the integration of information underpinning cognitive functions[2] remains to be elucidated.

1.2. The Physiology of Astrocytes

The critical role of astrocytes in the control and maintenance of ions, water, pH, neurotransmitters, and osmolyte concentrations in the extracellular space is well recognized.[31] Astrocytes can also release adenosine triphosphate (ATP), glutamate (Glu-), adenosine, D-serine, or γ-aminobutyric acid (GABA) through a process called gliotransmission.[32,33] Thus, besides their roles of housekeeper and neuron supporter, astrocytes also play active functions in brain processes occurring at multidimensional scales. At the nano- and micrometer scales, astrocytes are responsible for tightly controlling synapse formation, function, and

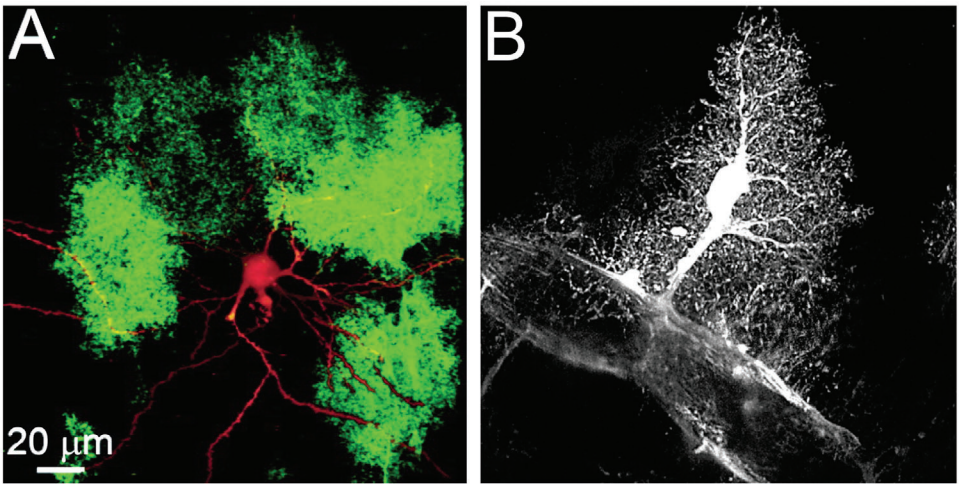

Figure 3. A) The two photon image shows a GFP expressing astrocytes (green stained cell) that surround and encompass many dendrites of the same neuron (red stainined cell), allowing an astrocyte to control multiple synapses. Reproduced with permission.[41] Copyright, 2007, Society for Neuroscience. B) Single plane two-photon confocal image of a cortical astrocyte expressing green fluorescent protein (eGFP). The high interlaminated wire of processes confers a polyhedral shape to astrocytes. Note an astrocytic process enveloping a small vessel. Reproduced with permission.[39] Copyright 2010, Elsevier.

elimination.[34] They are also responsible for mechanisms controlling the cerebral blood flow, which occurs through the astroglia syncytia at the cellular network scale.[5] Additionally, astrocytes provide metabolic support to neurons by acting as a syncytium to distribute energy substrates, such as lactate.[16] Supporting this role is clear genomic, molecular, and biochemical evidence that demonstrate distinct metabolic phenotypes between neurons (mainly oxidative) and astrocytes (mainly glycolytic).[35]

Recent in vivo evidence suggests that astrocytes play a functional role at the organ level, during cognitive processes such as memory and learning, and in particular points to a potential role of astrocytes in information processing.[2,32,33] Food intake, chemosensing, energy balance, and circadian rhythm[1,36] are the most recent functions affecting whole body function in which astrocytes have been implicated.

Here, we briefly summarize the most important functions of astrocytes, with the purpose of highlighting the molecular elements that were recently discovered and that might become the targets of glial interfaces, glial engineering, and glial photonics in the near future.

1.2.1. Homeostasis of Ions, Water, and Molecules in Astrocytes

The ionic, water, and molecular homeostatic processes in astrocytes are highly dynamic and are mediated by transmembrane proteins that form ion channels, water channels, and transporters, as well as receptors that are expressed on the astrocytic membrane in specific patterns and locations **(Figure** 4). Plasma membrane proteins allow both active and passive flow of ions, organic osmolytes, and osmotically driven water transport.

Molecular and functional events underpinning astrocytic homeostatic processes are spatially and temporally distributed.[31] The spatiotemporal displacement is caused by the variety of the proteins expressed, which differ not only in permeability, ligand selectivity, and gating properties, time-and voltage dependence profiles but also in expression pattern. It is now accepted that the expression of channel proteins is, in fact, polarized, patchy, and in some cases localized to specific regions in astrocytes, forming so-called functional microdomains.[13] Consequently, functional events might occur in or involve different astrocytic regions, such as the soma, process, or process end-feet. Moreover, since each astrocyte occupies nonoverlapping territory, each cell can be then conceived as a multifunctional unit that individually regulates distinct spatial areas in the brain.[32] This phenomenon, known as astrocyte segregation, may be crucial for normal brain function. Accordingly, in chronic conditions and neurodegenerative diseases—such as Alzheimer's Disease, depression, Amyotrophic lateral sclerosis (ALS), and/or after acute injuries such as ischemia, stroke or epilepsy—astrocytes lose the polarity of their process morphology and the specific spatial expression of channels and receptors,[6–8] with consequent loss of their homeostatic function. Thus, the expression and function of ion channels in astrocytes represent targets for novel devices aiming to better the understanding of brain function and for the treatment of a variety of neuropathologies.

1.2.2. Ion and Water Channels in Astrocytes

The main functional feature of astrocytes in vitro and in vivo is a large K^+ conductance, which accounts for the maintenance of astroglial membrane potential in the cell body and thus ensures many of the homeostatic astroglial functions.

In vitro and in vivo studies showed that astrocytes are equipped with a variety of voltage-gated potassium (K+) channels.[1,31] In particular, the potassium channels called delayed rectifying K^+ channel (K_{DR}), Two Pore Domains Potassium Channel (TREK and TWIK) and members of the inward rectifying K^+ channels (K_{ir}) family are responsible for the maintenance of resting membrane potential in astrocytes and for the homeostasis extracellular

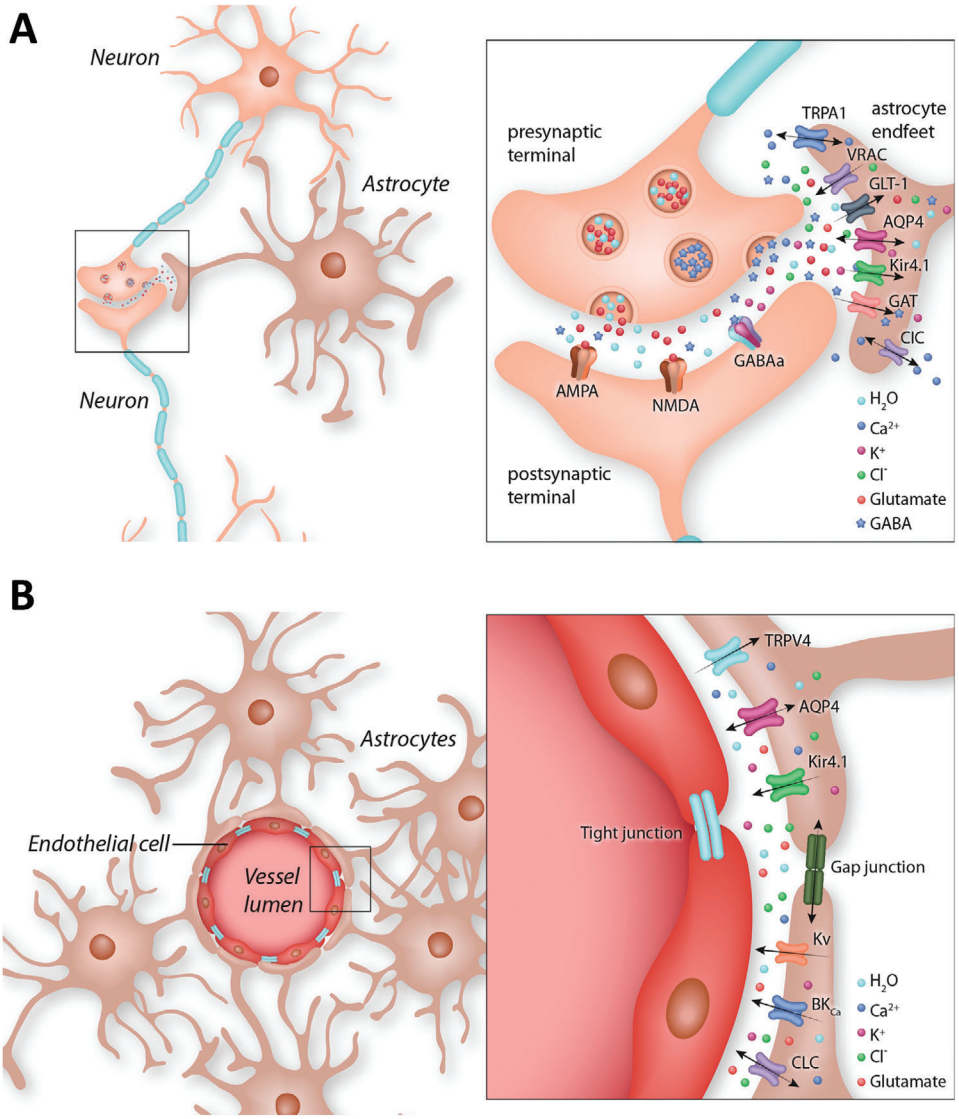

Figure 4. Scheme representing the expression of membrane proteins in astrocytes at distinct microdomains. A) At the perisynaptic astrocytic domain, after action potential occurrence and chemical synapse activation, neurotransmitters and K+ are released by neurons. Astrocytes maintain the concentration of Glutamate in the neuropil by uptaking it via Na+/Glu− co-transporter called GLT-1. The excess of K+ is buffered through the K+ channel Kir4.1. B) The gliovascular astrocytic domain. Gap junctions allow astrocytes coupling and the spatial redistribution of the uptaken K+ through the syncytium to reach the glia-vasculature interface. Voltage-gated K+ channels, 2-P domain K+ channels, calcium (Ca2+)-activated K+ (BKCa), and Kir4.1 mediate the release of K+ from astrocytes toward the vessels. Osmotically driven water movement is ensured by the water channels AQP4, which is majorly expressed at the endfeet of astrocytes which surround blood vessels. Chloride (Cl−) concentration is critical as this is the counterion that allow to keep electrochemical gradient homeostasis. Volume-regulated anion channels (VRACs) and members of the ClC family might contribute to chloride and volume homeostasis. Expression of the Ca^{2+} permeable channels TRPV4 and TRPA1 have been reported both *in vivo* and *in vitro*. Right panels in (A) and (B) represent zoomed-in view of distinct domains in contact with synapses A) and at the glia-vasculature interface B) Pumps and transporters that contribute to the transmembrane movement of ions have not been included.

K^+ concentration.[1,31,42–44] In particular, astrocytes' potassium permeability allows for the clearance of accumulated extracellular K^+ that occurs after an action potential. Astroglial cells uptake the efflux of K^+ ions resulting from neuronal activity. In this way, astrocytes counteract the possible increase in K+ concentration to detrimental levels.[42–44] By means of the Na+/K+ pump and K^+ channels, astrocytes redistribute excess K+ ions from the active neuropil and into the brain's extracellular fluids. Among K+ channels, the inwardly rectifying potassium channel $K_{ir}4.1$[23] is a weakly rectifying potassium channel with intrinsic voltage dependence regulated by Mg^{2+} ions and polyamines. Its gating properties depend on the reversal potential (E_{rev}) of K^+. Kir 4.1 is highly expressed in astrocytic endfeet, which contact synapses and blood vessels.[31,42] Its biophysical properties and patchy distribution allow for increase in inward K^+ currents in response to elevations of $[K^+]_o$, while weak rectification accounts for $[K^+]_I$ extrusion on distal process sites at the interface with blood vessels. The increase of Kir currents is inhibited by Cs^+ and Ba^{2+}.[24]

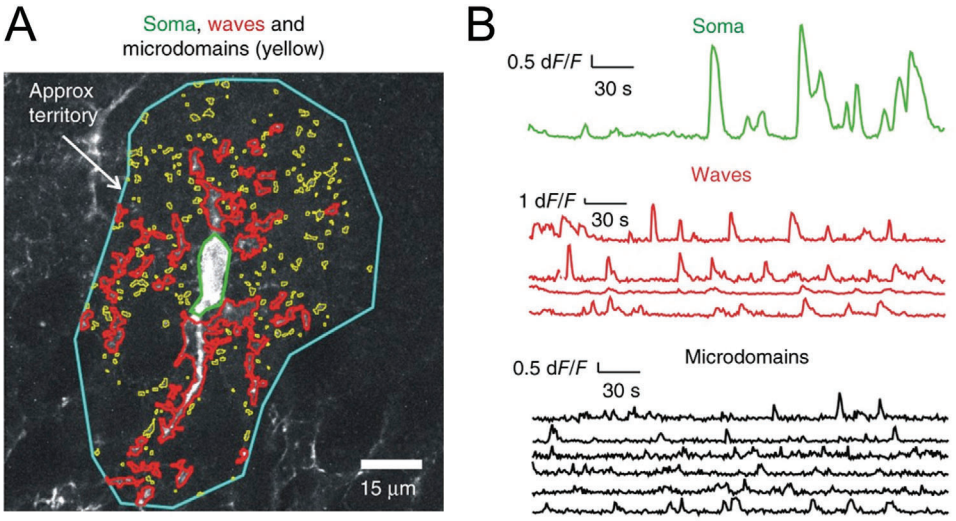

Figure 5. Calcium signaling in astrocytes A) Typical two photon image of calcium dynamics occurring in astrocytes microdomains (yellow), in astrocytic process (red), and the soma (green).[31] B) Representative traces of intracellular calcium variation observed in the respective regions. Adapted with permission.[38] Copyright 2015, Springer Nature.

Considering its significant contribution to the resting membrane conductance of astrocytes and to potassium buffering, Kir4.1 is a key element in astrocytic homeostatic processes and is essential for brain physiology.

Connexin-43 and 32 (Cx43 and Cx-32) are the main connexins expressed in both rodent and human astrocytes.[25–27] Connexins allow the flow of water ions and small molecules through the astroglial syncytia as well as between astrocytes and oligodendrocytes.[20–22] The functional existence of connexin hemichannels has been under debate for a long time. It is now evident that Pannexin-1, another channel protein belonging to the integrin proteins family, can form functional hemichannels. The physiological role of Panx-1 is unclear, but it has been implicated in the release of gliotransmitters ATP and D-serine release, and in the propagation of astrocytic Ca^{2+} waves.[21,38] Increases in extracellular $[K^+]$, membrane depolarization, ATP as well as mechanical stimulation can gate Panx1 channel pore.[21,38]

Besides potassium channels, astrocytes express a variable number of other channels (mainly calcium, chloride, sodium, and water channels) that account for astrocytic sensing, transduction, and responsive capabilities. These channels represent the molecular equipment that allows for the versatile responses of astrocytes to varied chemophysical stimuli (such as neurotransmitters, temperature and osmotic gradients, mechanical stimuli). Astrocytes can sense and respond to extracellular modification by oscillations in their intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$).[20,45–49] It has recently been highlighted that the dynamics of $[Ca^{2+}]_i$ are spatiotemporally distinct (**Figure 5**) depending on whether they occur at the soma, at their process in the so-called microdomains, or through the astroglial syncytia, by means of gap junctional coupling, originating what are known as Ca^{2+} waves (Figure 5C).[20] Astroglial $[Ca^{2+}]_i$ signaling occurs at astrocytic domains that enwrap the majority of pre- and postsynaptic cortical neurons in the tripartite synapse.[32,33,41] Of note, the speed of $[Ca^{2+}]_i$ propagation and waves increase with the evolution, further suggesting that astrocytic calcium signaling might have a role in computation.[2,4,53]

These findings led to the intriguing hypothesis that $[Ca^{2+}]_i$ are involved in and can become a therapeutic target for the improvement of cognitive functions.[2,50] In line with this hypothesis, aberrant Ca^{2+} signaling has been shown to be implicated in many pathological conditions, such as Alzheimer's Disease,[6,8] epilepsy,[51,52] brain tumors,[6,8] and ischemia.[8]

The entry of extracellular Ca^{2+} or the efflux of the Ca^{2+} stored in the endoplasmic reticulum both contribute to $[Ca^{2+}]_i$ oscillations and waves.[20] Among different paths, extracellular Ca^{2+} can flow through the membrane of astrocytes via the ionotropic purinergic receptor P2 × 7[54] and through Transient Receptor Potential Vanilloid 4 (TRPV4) and TRP Ankirin 1 (TRPA1).[45–49] TRPA1 was shown to regulate resting Ca^{2+} concentrations in astrocytes. Notably, TRPA1 mediates $[Ca^{2+}]_i$[45–48] oscillations occurring in astrocytic microdomains in situ that regulates astrocytic GABA transport, that, in turn can modulate GABAergic synaptic strength as well as the astrocytic release of D-serine. Interestingly, TRPA1 protein expression in human astrocytes has been recently demonstrated.[46] TRPV4 is a polymodal receptor that can respond to various stimuli, including temperatures >37 °C, osmotic stress, cell swelling, low ionic strength, volume changes, and agonists, such as 4α-phorbol 12,13-didecanoate, 4αPDD.[38,45,48,55–57] The channel pore of TRPV4 is nonselective for cations but displays an elevated permeability for Ca^{2+}. The TRPV4 protein is expressed in cortical astrocytes in vitro and ex vivo.[38,45,48,55–57] In the cortex, TRPV4 is localized preferentially in astrocytic endfeet.[45,47] A critical role for astroglial TRPV4 in the regulation of neurovascular coupling as well as in cellular and systemic hydro-saline homeostasis was demonstrated in vivo. The function or expression of TRPV4 is altered in pathological states, such as ischemia and stroke, both of which are characterized by imbalances in the brain volume.[58–60] The inositol 1,4,5-trisphosphate (IP_3) pathway, induced as a consequence of metabotropic receptors activation[20,38] or directly in response to

chemophysical stimuli, such as mechanical, osmotic stress,[20,38] majorly account for the release of Ca^{2+} from the intracellular stores. Nonetheless, recent findings showing the importance of mitochondrial calcium release have been demonstrated in vitro and in vivo.

Despite the physiological and pathophysiological importance of astrocytic calcium signaling, selective pharmacology is missing. Of note, a two photon imaging study in the mouse brain demonstrates that transcranial direct current stimulation (tDCS) can trigger astroglial $[Ca^{2+}]_i$ across the cortex.[61] Thus, astrocytic Ca^{2+} signaling represents an interesting target for device technology. In this regard, while the abundance of genetic tools to image calcium signaling is increasing rapidly, a major pitfall is the lack of devices enabling the selective evocation of Ca^{2+} signaling in distinct regions of the astrocyte.

Volume-regulated anion channels (VRACs) are anion permeable channels that are activated upon anisotonic challenge and that allow release of chloride and excitatory amino acids like glutamate, taurine, aspartate, and ATP.[62] After much research, the protein underpinning VRAC, called leucine-rich repeat-containing protein 8 (LRRC8), was recently identified.[62] LRRC8-A protein and VRAC current are expressed by astrocytes in vitro and in situ.[63–65] Notably, by a process called volume transmission, the volume-regulated release of excitatory amino acids from astrocytes into the area of the synaptic cleft could affect synaptic function.[66] In this context, LRRC8 might play a role in astrocytes ability to control synaptic function.

Voltage-gated sodium channels (Nav) are considered a hallmark of excitable cells. However, early patch clamp studies in astrocyte culture demonstrated the expression of both Tetrodotoxin (TTX) sensitive and TTX resistant currents in type 2 cultured astrocytes[67] and in spinal cord astrocytes. The expression of functional Nav was later confirmed in brain slices of the hippocampus and in the spinal cord.[68] It should be noted that the density of Nav protein and current is not sufficient to elicit action potentials, and thus the relevance of Nav channels to astrocytic physiology is still under debate. More recently, intracellular Na+ dynamics in astrocytes have been characterized in rodent brain slices. It is suggested that the Na+/Ca2+ exchanger accounts for the majority of observed Na+ oscillations. However, given the high permeability of TRP channels to Na+, the involvement of TRPs in extracellular Na+ influx[69] is indeed plausible. The functional significance of Na+ dynamics and waves are still unclear.[70] Their potential role in astrocytic homeostatic control of the extracellular environment as well as in the functional coupling between the metabolic needs of neurons and the metabolite supply of the vasculature has been suggested.[70]

Astrocytic control of water transport and the distribution of water throughout the brain is regulated by water channel aquaporin-4 (AQP4), which is expressed in astroglial foot processes, and enables astrocytes to rapidly control water homeostasis at the interface between brain tissue and blood vessels.[71,72] The dysregulation of AQP4 in pathologies such as epilepsy, brain tumors, or stroke is supported by substantial in vivo evidence.[71–73]

As microdomains are enriched structures devoted to specific functions, it is not surprising that a functional and molecular interplay has been described between water channels and ion channels. In particular, AQP4 has been proposed as molecular partner of the potassium channel Kir4.1, and this interaction accounts for osmotically driven water needed to balance ion movement during potassium spatial buffering.[74–76] TRPV4 cooperation with AQP4 is also critical for the ion and water dynamics that occur during volume regulatory mechanisms that react to osmotic shock in astrocytes[37,45,55] and ex vivo.[77] The functional interplay between AQP4 and VRAC has been demonstrated in rat primary astrocytes in vitro.[63] Moreover, the rise in the intracellular Na^+ concentration can inhibit VRAC currents in rat primary neocortical astrocytes and in adult astrocytes in rat brain slices. It was suggested that a biophysical interaction between cationic and anionic conductance might occur in brain astrocytes and that the Na+/Ca2+ exchanger and AQP4 are not involved in the observed effect.[78]

Astrocytes are also controlling the neurotransmitter homeostasis in the brain. The turnover of neurotransmitters, such as glutamate, GABA, adenosine, and norepinephrine, is regulated by their removal from the extracellular space via uptake through selective transporters and metabolic conversion through specific enzymatic pathways in astrocytes. Moreover, astrocytes can synthetize glutamine, a precursor for neuronal glutamate and GABA, and in turn affect both excitatory and inhibitory synaptic transmission between neurons.[1]

1.2.3. The Tripartite Synapse and Gliotransmission

It is now accepted that astrocytes play important roles in the regulation of synaptic transmission.[2,4,33,34,40,41] The endfeet of astrocytic processes embrace the presynaptic and postsynaptic neuronal terminals in a microdomain structure called the tri-partite synapse (**Figure 6**). Recent evidence indicates that such anatomic compartmentalization serves astrocytes' capabilities to sense and integrate synaptic activity by dynamic mechanisms involving the rise in intracellular astroglial Ca^{2+} levels and by astrocytic release of molecules (e.g., glutamate, D-serine, ATP, adenosine, GABA, Glycine). This process, called gliotransmission, can modulate neuronal excitability and synaptic transmission.[2,4,33,34,40,41] A selective transport patch has been identified for different transmitters, although in some cases, the occurrence of vesicle- or channel- release is still controversial.[2,33,34]

Notably, behavioral studies showed that bidirectional neuron–astrocyte cross-talk through calcium signaling, and that the tripartite synapse and gliotransmission are potential mechanisms underpinning memory modulation, sleep, and learning.[79,80] Moreover, astrocytes can shape the structure of synaptic networks by contributing to synaptic elimination and by regulating the volume of the extracellular space.[34]

More recently, a multipartite synapse model has further developed, which includes the ramified extension of microglial cells adjacent to the tripartite synaptic structure and the constituents of the extracellular matrix (ECM).

1.2.4. Astrocytes and Cognitive Functions

Since astrocytes have long been thought of as incapable of communication or any computing mechanism, the concept that astrocytes contribute to the information processing is rather new. Even more recent is the intriguing hypothesis that astrocytes can

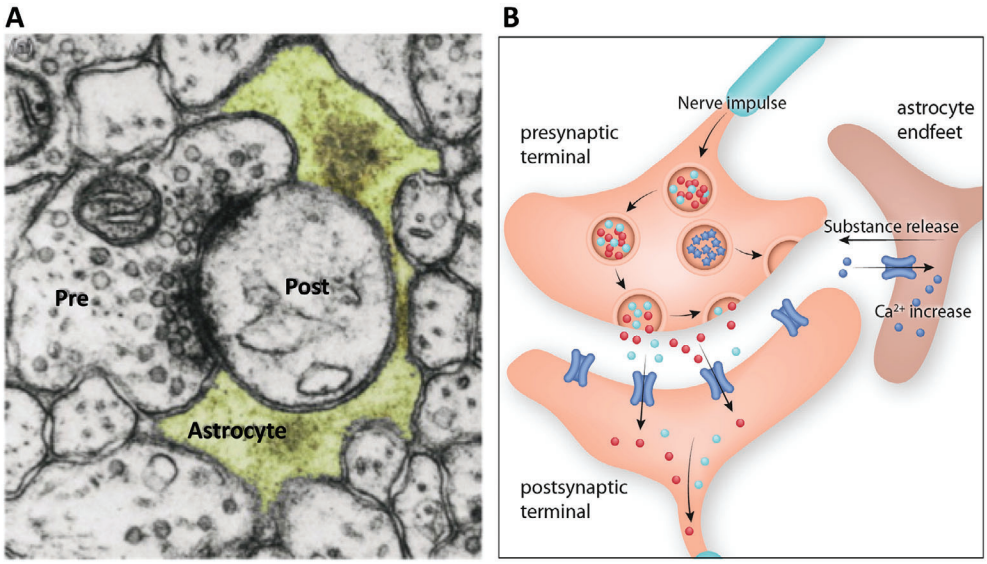

Figure 6. The tripartite synapse. A) Electron microscopy image of the tripartite synapse: the endfeet of an astrocytic process ensheathes a presynaptic (Pre) and postsynaptic (Post) neuronal terminal. B,C) Representative schemes of the image reported in (A). Astrocytes endfeet exert a critical function in the homeostasis of K^+ (K+ buffering), and of glutamate. C) Additionally, neurotransmitters released from presynaptic neuron can bind astrocytic metabotropic receptors and induce the a $[Ca^{2+}]_i$ increase in astrocytes cytosol from internal stores, by IP3 pathway. The $[Ca^{2+}]_i$ signal promote the release of gliotransmitters from astrocytes endfeet, providing bidirectional communication among tripartite synaptic components. Spatial location of astrocytic transporters and receptors is not representative of their exact spatial distribution. Panel (A), Adapted with permission.[80] Copyright 2007], Elsevier.

integrate neuronal functions at the synaptic and network levels and that their function can influence behavior.[2,4,80] According to the fact that astrocytes occupied 20-fold larger volume in the human brain than that in rodents, it was plausible to speculate that astrocytes can participate in or influence information processing in specific ways by integrating and computing data.[4] Even though we are far from proving that astrocytic computation is causally linked with the human intelligence, evidence for the role of astrocytes' function in the mechanism of memory formation and learning has been demonstratedadifferent experimental models.[2,14,80]

Pathophysiological data also support the importance of astrocytic ion channel and water channel dysfunction that is observed in diseases characterized by cognitive impairment. KCNJ10, the gene encoding the potassium channel Kir4.1, has been linked to developmental disorders like autism.[58] Moreover, alteration in Kir4.1 current amplitude and the consequent astrocytic dysfunction have been observed in traumatic injury, ischemia, and in pathologies characterized by generalized neuroinflammation. Additionally, neurodegenerative diseases such as Alzheimer's Disease and ALS implicate loss of Kir4.1.[81–83] Recent evidence accounts for a functional role of AQP4 in synaptic plasticity, learning, and memory. In vitro and in vivo studies using AQP4-null and wild-type mice, in particular, show the impairment of long-term potentiation observed in the hippocampus. These findings were accompanied by behavioral studies that may shed some light on a specific role of AQP4 in memory function.[84] Moreover, the implication of TRPA1 receptors in the detrimental changes to memory that occur in the elderly has been recently reported.[85] Although the physiological role of TRPV4 is unclear, its role in brain pathologies and injuries characterized by inflammation and impairment has been well defined.[59,60] Recently, the relevance of TRPV4 in cerebrovascular function was highlighted when researchers found an impairment in the cognitive function of mice lacking the channel protein.[86,87] Several lines of in vivo evidence indicated that astrocytes can modulate or enhance neuronal oscillations and synchronization, which are both important mechanisms for memory formation, learning, and sensory perception. Activation of astrocytes can also coordinate the activity of neuronal networks and their transition from desynchronized states to synchronized, oscillatory states.[2] In particular, in vivo studies showed that noradrenergic stimulation of astrocytes can result in the generation of slow wave oscillations that are important for cognitive tasks, such as memory consolidation. It has been hypothesized that activated astrocytes can release glutamate in a defined extracellular domain that in turn triggers cooperative, synchronous neuronal firing and generates slow waves.[2] Interestingly, the ability of astrocytes to generate slow wave oscillations has been recently demonstrated by means of nanostructured devices (see Section 2.1 for details).[88] Thus, the possibility of a direct involvement of active astrocytes in the generation of slow wave oscillations cannot be ignored.

2. Smart Glial Engineering and Interfaces for Measuring and Probing Astrocytes

Astrocytes cannot generate action potentials. However, as described above, astrocyte membrane proteins can form channels and receptors that generate ionic (e.g., ion flux generating a current, intracellular Ca^{2+} changes) and molecular (e.g., water flux) signals, and these signals can be captured by custom tailored

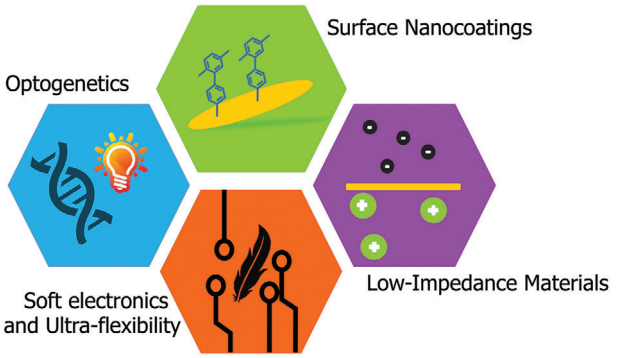

Figure 7. The key properties of glio-interfaces. A brief schematic of the key features needed to develop a new class of tools enabling investigation of astrocyte–astrocyte and astrocyte-neuron interactions.

electronic devices. Moreover, recent literature has shown that astrocytes can sense and respond to mechanical, electrical, and photonic stimuli by activating ions and water transport as well gliotransmitter release. To this end, specific features must be integrated into probing technologies in order to guarantee the suppression of noise, the discrimination between neuronal and astrocytic signaling and the interrogation of specific pathways in cells. We call these properties glio-interfacial key properties (see **Figure 7**). We believe that ultraflexible interfaces and soft electronics together with dedicated nanofunctionalization of surfaces and low impedance materials can offer a unique toolset to unveil this secret language between astrocytes and neurons both in healthy and pathological conditions.

Here, we report on technologies engineered to study astrocytes by means of electronic, photonic, and biomaterial approaches, and according to these recent findings, we present a new perspective to investigate astrocyte functionality (**Figure 8**).

Wanke et al. show a direct interaction between spike trains and glial response through a sensing electrode.[89] Moreover, according to the results of Fleischer et al., even microelectrode arrays can be used to detect astrocytic extracellular currents after cell stimulation.[90] However, the difficulty in revealing such signals in astrocytes is due to the very diverse nature of these fluctuations both in amplitude and in frequency in comparison to neuronal activity.

To address this challenge, there is a need for sensitive devices capable of detecting small, low frequency, slow, and long-lasting voltage membrane variations. Micro- and nanostructured materials can be helpful for tackling this challenge since they are intended to record small amplitude and low frequency signal expected from astrocytes. Indeed, they ensure close contact of the device with the cells or even allow for direct engulfment of the material by the cell that decreases the cleft distance between the cell and the device. This therefore enables a more efficient cell-to-device coupling with a consequential increase in the signal to noise ratio (S/N).[91] In particular, recently, microstructured mushroom-shaped electrodes have been proposed by Mestre et al.,[92] to perform extracellular recording in primary astrocytes. The authors observed spontaneous burst in astrocytes, comprised of quasiperiodic signals, with a frequency of ≈0.1 Hz and with a broad distribution in amplitudes that vary from 10 to 60 µV, preceded by an increase on the average noise fluctuations. Moreover, Saracino et al.,[88] have demonstrated the efficacy of gold coated silicon nanowire devices (Au/SiNWs) in recording extracellular currents from primary astrocyte in vitro. The features of the signals detected by Saracino et al.,[88] displayed variable amplitudes from 17 to 132 µV and duration from 60 to 730 ms with interval between two distinct events occurs in a range from 0.2 to 26s. The power spectral density analysis showed that signals recorded from astrocytes grown on Au/SiNWs occurred in the frequency ranges (δ (0.1–5 Hz, θ (5–9 Hz), and β (9-30 Hz). The same work also shows how the dendritic nanotopography of the nanowires promotes astrocyte adhesion and in vivo like differentiation, acting at the same time as smart scaffold and smart electrode without the need for any biochemical functionalization. Notably, the authors showed that the extracellular signals can be recorded only in differentiated astrocytes on Au/SiNWs.

Such functional properties of the cells are inherently linked to the disordered topology of inorganic nanostructured materials that resemble the neural and glial thread-like structures in the brain. Indeed, disordered silicon nanowire-based devices have already been proposed as active biointerfaces for the growth and the treatment of different cells (e.g., colon cancer cells) and smart biosensors.[93,94] The properties of astrocyte signals described above can be targeted to segregate astrocyte signals from neuronal action potentials.

It is important that the morphological differentiation was accompanied by a more mature functional phenotype: astrocytes on the nanowires were more hyperpolarized and displayed a higher resting permeability and capacitance as well as increased expression and function of the potassium channels Kir4.1 (**Figure 9**).

These findings are also confirmed by the implementation of other inorganic nanostructures, such as Hydrotalcite-like compounds (HTlc) that are layered materials consisting of positively-charged layers and exchangeable interlayer anions (Figure 9A). Such elements can be used in different configurations from nanoparticles to microsheets, and they exhibit interesting properties in promoting astrocyte morphological differentiation by inducing cytoskeleton rearrangement.[95] Similar to the observations of astrocytes on Au/SiNWs, astrocytes on HTlc, displayed large inward conductance with biophysical features of Kir4.1 as well as upregulation of the protein across the whole cell as well as in the plasma-membrane. It should be noted that AQP4 expression and water permeability were upregulated in astrocytes grown on HTlc (Figure 9D). In summary, astrocytes grown on HTlc and Au/SiNWs recapitulate the structure and the main homeostatic function of astrocytes that are lost by growing these cells according to the Mc Carty and de Villis method.[96]

In this view, these glial interfaces provide an alternative path to study astrocytes in a more in vivo like state, with the benefit offered by having a controlled in vitro environment.

These materials together with ultracompact, high performing electronics can be a successful combination of technologies to uncover the chattering of glial cells and their role in physiological and cognitive mechanisms. To this end, customized low-impedance amplifiers with low latency can be crucial to investigate glial behavior under specific stimulation patterns.[97] To date, most of our knowledge on the neuron-glia interaction is limited to the single-cell level or to neuronal activity in networks. In contrast, much less is known about the mechanisms underpinning neuron-astrocyte crosstalk at higher-level complexity,

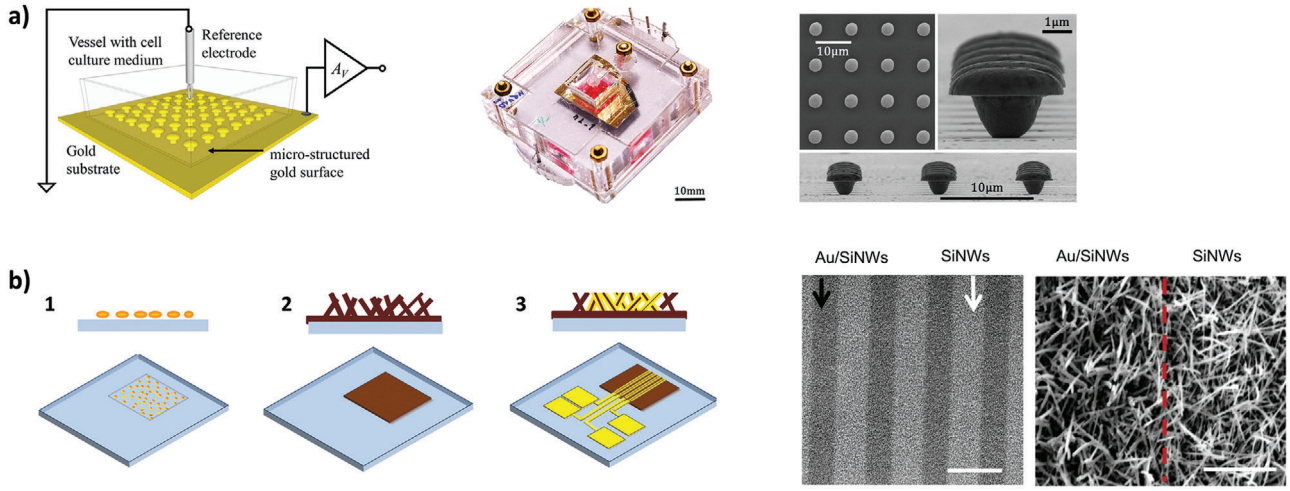

Figure 8. Micro and nanostructures to increase the astrocytes' SNR and to detect their extracellular signaling: a) A sketch of the device with electrical connections and the real biosystem. On the right a detail of the gold mushroom-like structures. Adapted with permission under the terms of the Creative Commons CC BY license.[68] Copyright 2017, the Authors. Published by Springer Nature. b) A sketch of the fabrication steps for a nanoelectrode based on disordered silicon nanowires coated with gold. On the right part a detail of the electrodes and a photo of the underlying nanostructure. Reproduced with permission.[88] Copyright 2020, Wiley-VCH GmbH.

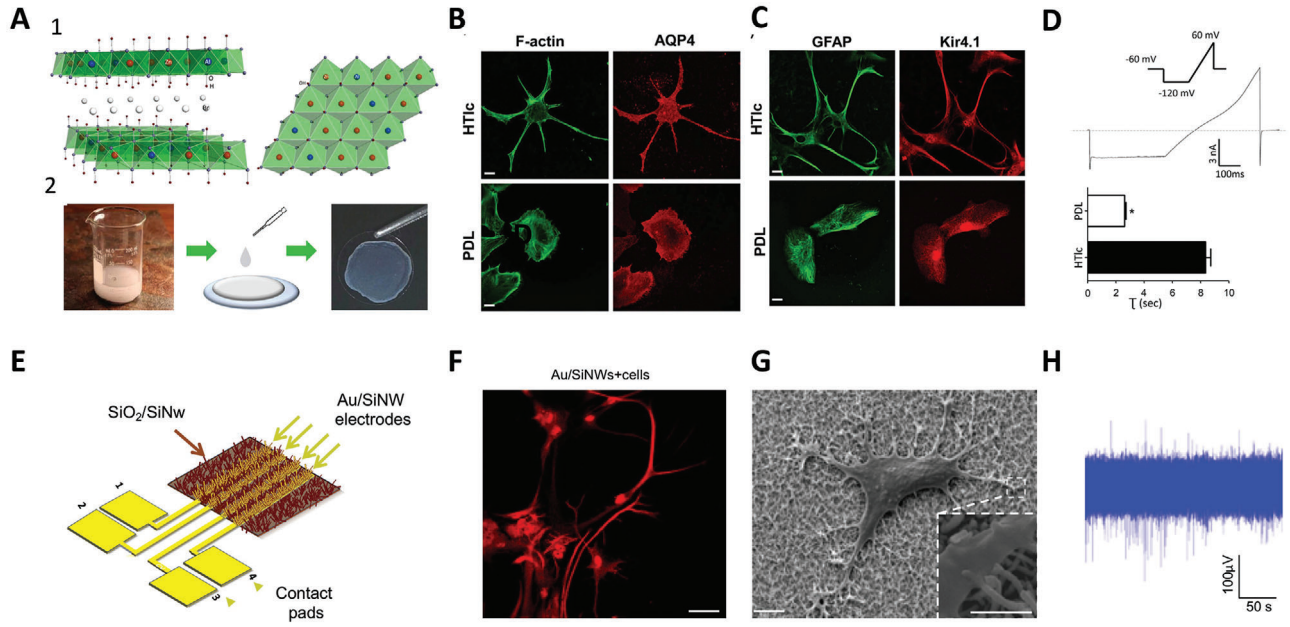

Figure 9. Nanostructured glial interface. A) 1) Schematic representation of structure 1) and preparation 2) of ZnAl-HTlc films. B,C) Confocal images of astrocytes grown on HTlc NPs (upper panels) stained for AQP4 or Kir 4.1 (B, C, red), and Actin or GFAP (B, C, green). Note the intense differentiation of astrocytes on HTlc. D) Functional properties of astrocytes grown on HTlc showing inward conductance (top) in response to ramp stimuli and increased the swelling rates (τ, bottom). E) Scheme of Au/SiNWs Micro Electrode Array (MEA). F) Confocal imaging of astrocytes stained for Kir4.1 on Au/SiNWs G) Scanning Electron Microscopy (SEM) micrographs of a differentiated astrocyte plated on Au/SiNWs. Inset: higher magnification images showing astrocytes process enveloping a nanowire. H) Extracellular recording obtained from astrocytes grown on Au/SiNWs MEA. A–D) Reproduced with permission under the terms of the Creative Commons CC BY license.[95] Copyright 2016, the Authors. Published by Nature Publishing Group. E,F) Reproduced with permission.[88] Copyright 2020, Wiley-VCH GmbH.

i.e., neuron-astrocyte networks. The natural evolution of these nanostructured electrodes is their application to astrocyte-neuron cocultures and in brain tissue in order to discriminate the different signals and therefore shed light on the basic mechanisms underlying astrocyte-neuron communication. This perspective could finally elucidate the real physiological meaning of altered function of glial cells and explain the numerous neuropathological states in which astroglial cells are implicated, such as epilepsy, spreading depression, Alzheimer's disease, Huntington's disease.

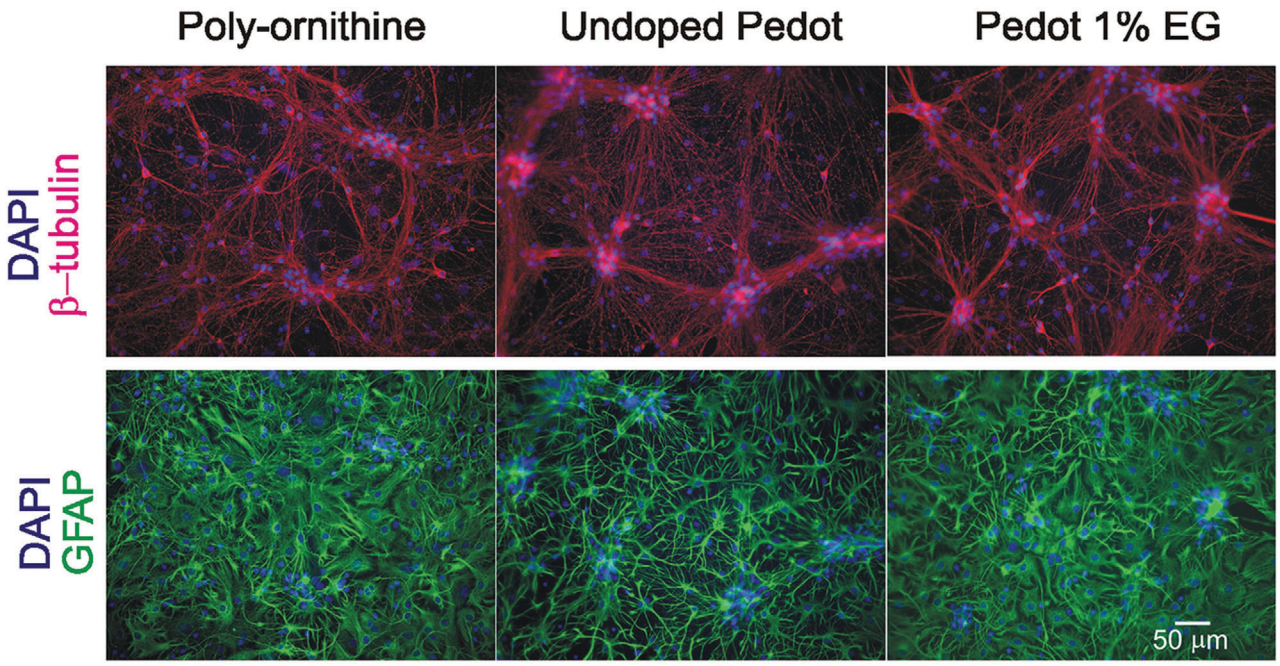

Figure 10. Immunofluorescence images of primary hippocampal cultures of neurons, stained for β-tubulin (red) and astrocytes, stained for GFAP (green), grown on PEDOT:PSS 1% EG, and PEDOT:PSS 3% EG substrates. Reproduced with permission under the terms of the Creative Commons Attribution License (CC-BY 4.0).[103] Copyright 2015, the Authors. Published by Frontiers.

2.1. Organic Bioelectronic and Optoelectronic Glial Interfaces

Organic semiconductor or conducting polymers display a combination of intrinsic properties, such as electrical conductivity, long-term biocompatibility, mechanical flexibility, and adaptable form factor, that are advantageous with respect to traditional silicon-based technologies.[75,76] Among bioelectronic polymers, (poly(3,4-ethylenedioxythiophene) polystyrene sulfonate) (PEDOT:PSS), polypirrole (PPy), and polyaniline (PANI) can increase ion current, as well as electron and hole transport, thereby offering a broad spectrum of possibilities to stimulate and to record the function of neural cells in vitro and in vivo.[98–102]

The biocompatibility of PEDOT:PSS-based substrates has been investigated with hippocampal primary cultures of neurons and astrocytes.[103] The authors analyzed the electrical and morphological properties of PEDOT:PSS doped with different concentrations of Ethylene glycol (EG), and found that the increasing concentration of EG that can proportionally rise the conductivity of the substrate. Moreover, in agreement with findings observed using other material interfaces,[104] the signaling of neurons in terms of adhesion and outgrowth was the opposite to that observed in primary astrocytes. In particular, hippocampal neurons survive and exhibit functional properties comparable between PEDOT:PSS and controls. On the other hand astrocytes' growth was reduced with increasing EG concentration in PEDOT:PSS samples (**Figure 10**).

Thus, by increasing EG, selective reduction of glial cell reactivity can be achieved, while increasing conductivity of the organic polymer without influencing the functional properties of neuronal networks.

It is worth noting that the first report on organic glial interfacing devices used astrocytes as the cell type of choice. Berggren's group[105] used astrocytes because of their elevated expression of glutamate receptors to validate the ability of organic ion pump to release neurotransmitters. The ion pump was placed at the close proximity of primary mouse astrocytes and loaded with glutamate (Glu) at the source and with NaCl electrolytes at the cathode.[105] The authors verified that astrocytes grown on the organic ion pump display $[Ca^{2+}]_i$ increase upon binding of Glu and release of it by the pump. Interestingly, the same ion pump approach was later used to deliver the inhibitory neurotransmitter, γ-aminobutyric acid (GABA), to switch off status epilepticus locally with a spatial resolution of micrometer precision.[106] Notably, the organic electronic ion pump technology was integrated into the recording site electrode and thus delivers GABA immediately in response to the recording of epileptic activity. GABA delivery stopped epileptiform activity in the location in which it was delivered, which was shown through simultaneous recording. However, the eventual role of the released GABA molecule on the astrocytic contribution to epileptic activity has not been investigated. Several lines of evidence support the tenet of astrocytic involvement in epileptic electrogenesis. The physiological basis of astrocytic K+ and glutamate/GABA hypotheses of epilepsy have been provided for in vivo animal models and in humans.[107]

The use of ionic pumps to release neurotransmitters in a spatially distributed manner could be very helpful for exploring the impact of these neurotransmitters on astrocytic microdomains. This type of study might be relevant in providing insight into the pathogenic and/or reactive mechanism of astrocytes in epilepsy. Moreover, this approach might provide clues to the long-lasting debate regarding if astrocytic activity occurs independently from neuronal activity. Finally, the ability to precisely sense the amino acids released by astrocytes during gliotransmission presents a

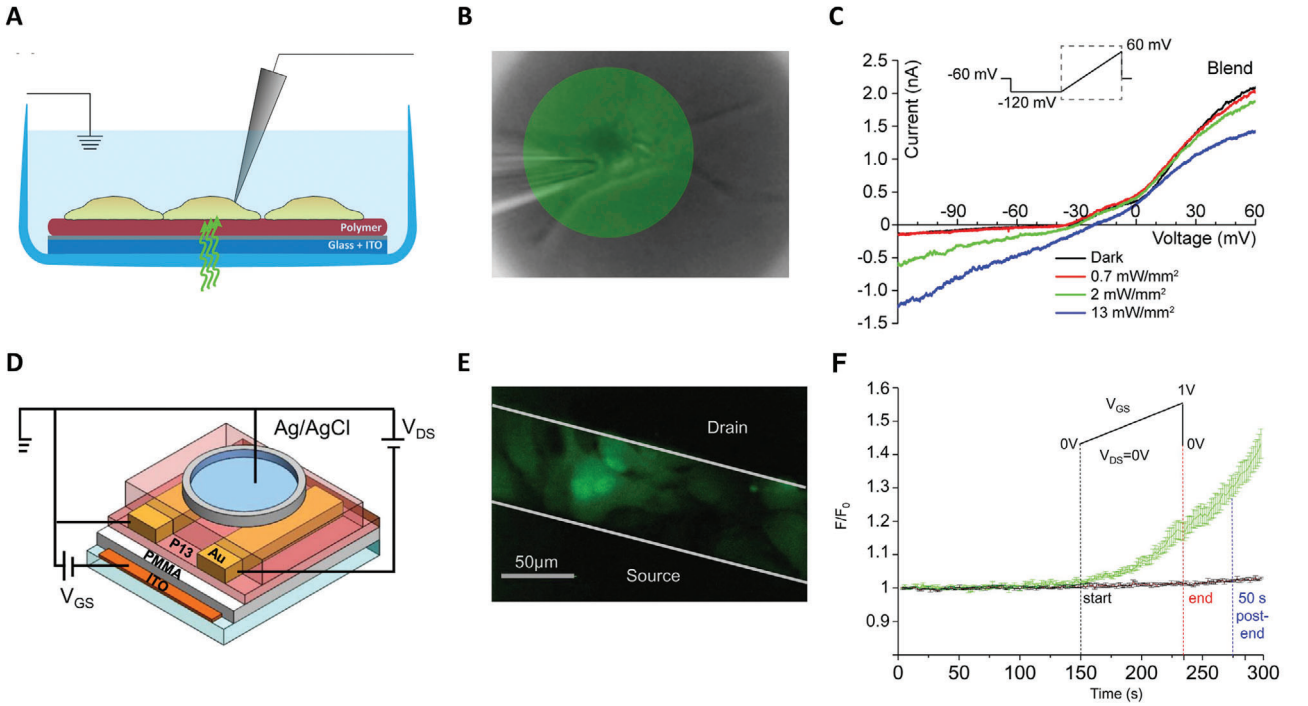

Figure 11. Organic bioelectronic and optoelectronic glial interface. A–C) Scheme of P3HT:PCBM based device a) enabling photoexcitation with visible light b) and eliciting ionic current in primary astrocytes c). Reproduced with permission.[111] Copyright 2014, Wiley-VCH GmbH. B,D–F) Scheme of P13 based device a) enabling extracellular electrical stimulation and calcium imaging of fluo-4 loaded primary astrocytes e). The electrical stimulation evokes calcium signaling in primary astrocytes c). Reproduced with permission.[114] Copyright 2020, Wiley-VCH GmbH.

major challenge to bioengineering and glial engineering technology since no solution to this technological challenge currently exists.

In particular, specific devices need to be conceived to elicit biochemical and electrical signals in astrocytes. In this context, Benfenati & Lanzani et al., pioneered and developed an approach based on the photoexcitation of a thin film polymer photovoltaic blend, formed by mixture of poly(3-hexylthiophene) with phenyl-C61-butyric-acid-methyl ester (P3HT:PCBM), that allows for neuronal firing in vitro and can restore visual capabilities in a pathophysiological mouse model of the blind retina.[108–110]

In collaboration with Antognazza,[111] it has been demonstrated that P3HT:PCBM supports the growth of pure primary cultured rat neocortical astrocytes (**Figure 11**A). Astrocytes grown on P3HT:PCBM displayed comparable electrophysiological properties with those of cells grown on standard substrates. Notably, photoexcitation of the P3HT:PCBM blend with 543 laser light in the range of few mW mm^{-2}, can trigger the whole-cell membrane conductance of the excited astrocyte and depolarized its membrane potentials. The effect was directly proportional to the light excitation density. The biophysical and pharmacological analyses suggested that the ClC-2 protein channel mediated conductance is critically involved in the observed effect, and proved that photostimulation of the device could be used as a tool to uncover the role of astrocytes in brain function and dysfunction. Regarding the mechanism, it was proposed that acidification of the extracellular pH, hypotonicity-induced cell swelling and alteration of F-actin cytoskeleton were possibly involved in the observed effect.[111] Additionally, recent work from our lab and the Mahadevan-Jansen group showed that infrared pulsed stimulation of primary cortical astrocytes induced a TRPV4 and TRPA1-mediated rise in calcium signaling and activated water transport along with the consequential change in cell volume.[112] Similarly, studies in neurons also identified TRPV1 as one of the possible players involved in the neuronal response to the blend-mediated photostimulation.[113] Thus, it would be interesting to explore if P3HT:PCBM blend photostimulation might perturb calcium signaling, cell volume regulation mechanisms as well as actin dynamics in astrocytes. While the nongliotic impact of P3HT:PCBM blend-based retinal implant has been demonstrated in situ,[87] the effect of P3HT:PCBM blend photostimulation in vivo as a gliophotonic interface for cortical astrocytes or for Müller glia in vivo has been completely unexplored.

Among small molecule organic semiconductors, perylene dimmide derivative *N*, *N*'-ditridecylperylene-3,4,9,10-tetracarboxylic diimide (P13), has been shown to be biocompatible with rat primary astrocytes in vitro.[114] In a recent work, it is shown that transparent organic cell stimulating and sensing transistor (O-CST) architecture, fabricated with P13,[115] was used to explore the impact of field effect extracellular stimulation on intracellular calcium concentration ($[Ca^{2+}]_i$) in primary rat neocortical astrocytes. Calcium imaging experiments enabled by the transparency of O-CST showing that a slow rise in extracellular potential between the gate and the grounded source electrodes provide an effective extracellular electrical stimulation of astrocytes that induces a slow and persistent increase in astrocytic $[Ca^{2+}]_i$. Pharmacological analyses demonstrated that TRPV4 and TRPA1 largely mediated the extracellular $[Ca^{2+}]_i$

increase. The O-CST device architecture was important to provide effective stimulation and $[Ca^{2+}]_i$ excitation in astrocytes. An additional noteworthy finding is that the extracellular field application induces a slight but significant increase in the cell volume. Nonetheless, the stimulation protocol applied to the astrocytes failed to evoke action potential in primary dorsal root ganglion neurons in vitro.[114,115] Collectively, the results reported in the study highlight that organic bioelectronic devices are promising glial interfaces that can selectively excite and control the physiology of astrocytes.

2.2. Carbon Nanotubes

Carbon nanotubes (CNTs) and their derivatives are promising biomaterials for use as scaffolds to drive nerve regeneration in neuroregenerative medicine, given their ability to modulate neurite extension and improve the conduction capability of neurons.[116] The work performed by Parpura's group[117,118] showed that chemically functionalized single-walled CNTs (SWCNTs) used as colloidal solutes or coating films of glass coverslips can alter the morphological and functional phenotype of primary rat astrocytes. In particular, unlike pristine CNTs, astrocytes on functionalized SWCNTs or multiwalled CNTs (MWCNTs) display better adhesion and distinctly-shaped morphology, which varies depending on the chemical modification and composition. Notably, glial fibrillary acidic protein (GFAP) immunoreactivity differed depending on the compound used.[117,118] The authors questioned whether GFAP might be involved in the astrocytic response to material interfaces and proposed it as a target of study. Based on results from GFAP knockout mice, they demonstrated that GFAP is a critical molecular structure underpinning the mechanism involved in astrocyte death, induced by the CNT.[117,118] In more recent work, the same group showed that SWCNT-polyethylene glycol (PEG) enhances astrocytes' ability to remove glutamate from the extracellular space by increasing expression of glutamate transporter (GLT) in the cell membrane, which was paralleled by an increase in GFAP immunoreactivity. The authors also discussed that astrocytes exposed to SWCNT–PEG became larger and stellate, morphological features of maturation, and/or of reactive astrocytes. The differentiation observed was not remarkable as in other following studies using different materials,[88,95] but the work suggests that carbon-based materials and nanostructured interfaces might be powerful tools for glial interfacing technologies aimed at actively modulating astrocytic structure and function.

2.3. Graphene

In the recent years, graphene has been proposed as promising material to be used in implants and neural devices for the modulation of physiological activities or even recovering altered brain functions.[119,120] Nevertheless the biocompatibility of graphene is still controversial since it can interfere with the exchange of ions and, more in general, with cell functionalities.[95–97] In case of astrocytes, a reliable graphene-derived interface should enhance cell growth and differentiation, avoiding reactive astrogliosis.

Defteral et al.[121] found that thermally reduced graphene (TRG) flakes injected into the adult olfactory bulb did not alter de novo neurogenesis. Moreover, these materials do not modify astrocytes survival and do not trigger inflammatory response. In this case, TRG may be used as viable way to interact with glial cells, exploiting their potentiality in repairing the impaired tissue. 3D graphene foams (3D-GFs) have also emerged as a novel scaffold with a specific property of differentiating neural stem cells into neurons. Along the same line of work, the preparation and exploitation of two GFs with different stiffness as neural interfacing scaffolds has been investigated, including the effect of GF mechanical properties on different parameters of neural cell physiology.[122] It has been highlighted as stiffer scaffold could boost neural stem cells adhesion, growth, and differentiation toward astrocytes compared to the softer substrates.

The exposure of primary cortical astrocytes to micro- and nanoflakes of graphene (GR) and graphene oxide (GO) did not decrease the cell viability even after seven days of incubation.[123] However, astrocytes incubated with graphene and GO flakes exhibited remarkable changes in their morphology with multiple protrusions similar to differentiated astrocytes in vitro. As shown previously by our group using first HTlc and later Au/SiNws,[88,95] Chiacchiaretta et al.,[123] also observed that treatment with GO flakes induces a significant alteration of K^+ currents along with an increase in outward rectifying currents. In alignment with alteration of Kir currents, the passive membrane properties of astrocytes incubated with GO flakes, which included a hyperpolarization of the resting membrane potential, a decrease in their input resistance and an increase in the specific conductance, were also altered. Additionally, they found that astrocytes treated with GO flakes had enhanced Na^+-dependent glutamate uptake capabilities as well as displayed alterations in calcium signaling. It was suggested that these effects were linked to alterations in the lipid content of the cell membrane caused by GO-flakes.

Considering the controversy raised by graphene flakes, the group of Palermo proposed to chemically change GO with a synthetic phospholipid (PL) to favor the interaction between GO and astrocytes.[124] To this end, PL moieties were linked on GO sheets and the resulting substrate was used as bidimensional scaffold for growing primary rat cortical astrocytes. A significant improvement results from this modified material, with an improved adhesion (about three times) if compared to that on glass substrates coated with standard adhesion agents (i.e., poly-*D*-lysine, PDL) or with respect to that on nonfunctionalized GO. Additionally, GO–PL did not display marked astrogliosis, as assessed by GFAP staining, thus demonstrating that GO–PL did not induce an inflammatory response at least for their interaction with astrocytes in vitro.[124] Thus, GO–PL grafting might be useful for neural prostheses to improve permissive cell colonization and limit glial scar formation. Moreover, this improved adhesion could support specific devices enabling neural cell sensing or interfaces that can tune physiological activity of astrocytes.

Even with these important findings, the use of graphene as a glial interface is still limited. Indeed, at present, graphene-based materials or devices designed to directly target astrocytic function or structure are lacking.

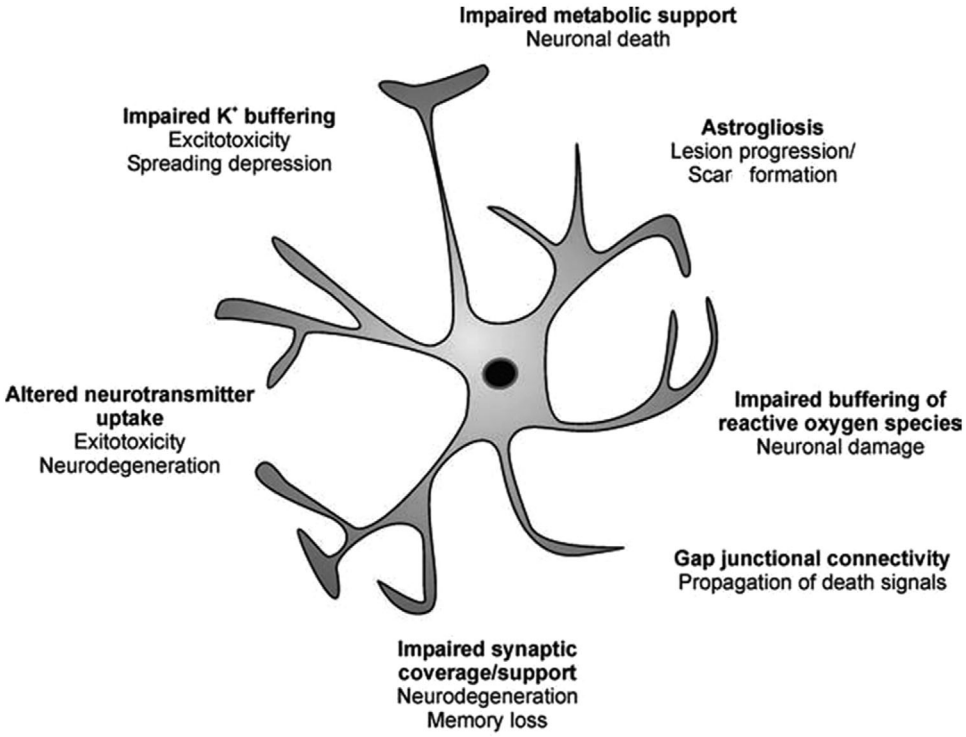

Figure 12. Schematic draft of the pathological potential of astrocytes. Adapted with permission.[6] Copyright 2008, Academy of Sciences of the Czech Republic.

3. Astrogliosis and Gliopathologies

The pathological potential of glial cells is a well-accepted fact. Any kind of brain insult does indeed affect astrocytes' structural and functional properties (**Figure 12**), and how astrocytes respond to the insult is believed to determine the survival of the brain parenchyma, the severity of the damage and, in turn, the balance between neurological defect and functional abilities of the patients.[6–9]

Gliosis refers to a variety of structural and functional changes that occur in glial cells, particularly in astrocytes and microglia, in response to chronic or acute insults to the CNS. The magnitude and kind of alterations that occur in astrocytes are variable and depend on the type of pathology and/or on the severity of the injury. Common features of gliosis include hypertrophic cellular proliferation; upregulated expression of several proteins, including GFAP and vimentin in astrocytes; presence of cytokines, chemokines, and growth factors; and ultimately the formation of a persistent fibrous scar.[125]

It has long been debated whether gliosis provokes beneficial or harmful effects on the surrounding cellular content. Uncontrolled reactive gliosis causes a massive neuroinflammation that leads to neuronal death and tissue damage. As an example, epileptic seizures can be triggered by genetically induced astrogliosis in mice without any other neuropathology.[126] However, reactive astrocytes could assume a neuroprotective or neurotoxic fashion depending on the cross-talk with microglial cells, which critically activate a subtype of astrocytes that in turn contribute to the death of neurons and oligodendrocytes in neurodegenerative disorders.[127]

The biochemical signaling cascade, molecular paths, and structural changes observed in reactive astrocytes are very complex and have been recently reviewed.[82] This recent work emphasized that the alteration of expression and function of ion channels[128] and transporters is believed to critically compromise the physiological abilities of astrocytes, which consequently leads to changes in neuronal excitability and to detrimental neurodegenerative processes. In particular, dramatic changes in the membrane expression and/or gain/ loss of function of inwardly rectifying potassium channel Kir4.1, excitatory amino acid transporter 2 (EAAT2), the gap junction protein connexin 43 (Cx43), the calcium channel TRPV4 and of the water channel AQP4 have been abundantly reported in pathological animal models and in humans.[6–9,83–85,128]

Since astrocytes are an integral part of brain circuits, constantly interacting with neurons and blood vessels, it is important to develop materials and technologies that are able to interface with astrocytes and that are compatible with technologies already available for interfacing with neurons. Designing glial interfacing devices with these considerations in mind will allow scientist to generate a platform to gain new insight into brain function. Nonetheless, glial interfaces might represent alternative therapeutic opportunity for the treatment of brain disorders that have thus far proved elusive. In this view, a large and exhaustive literature reports the different steps of the gliosis process usually triggered after an implant insertion.[10,131–133]

The natural response of the brain to the implantation of a device does not differ from the common gliotic process observed upon traumatic injury (**Figure 13**). After insertion of a device, inflammation and encapsulation occur together along with a

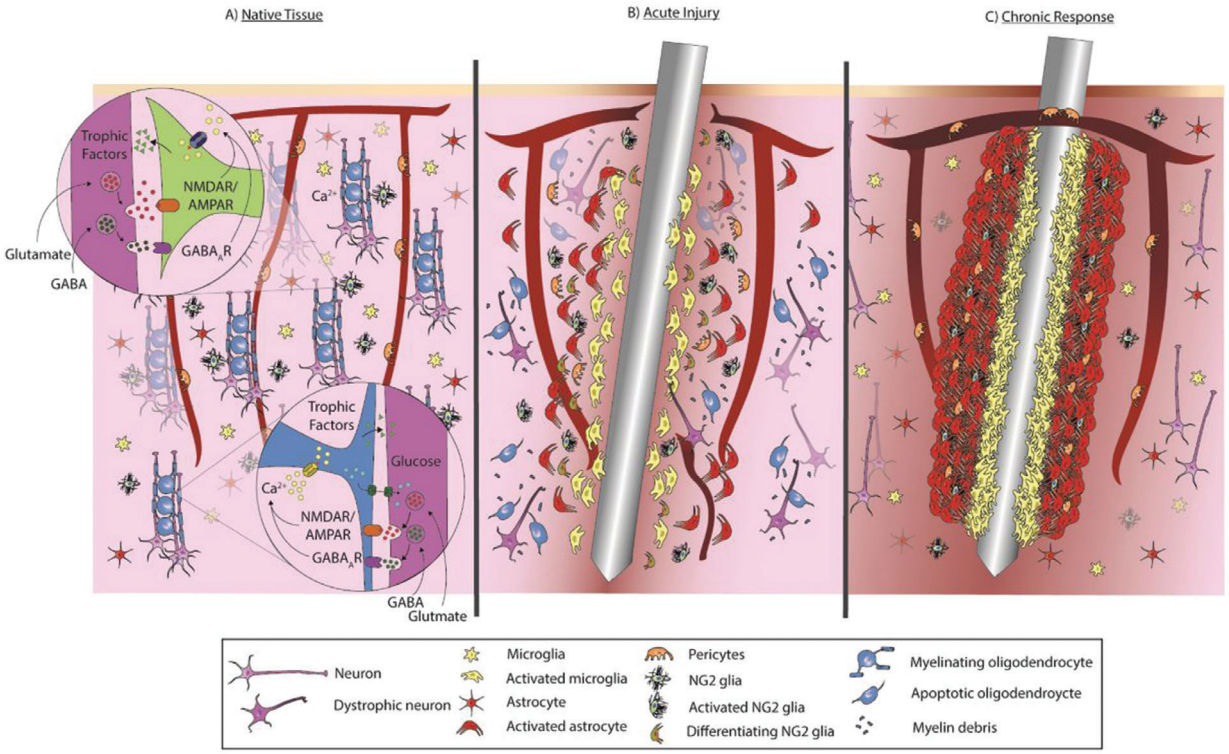

Figure 13. A schematic draw representing the foreign body response to implantable devices in the brain tissue. A) All the different types of cells with their functions in the healthy brain tissue; B) A scheme showing cell recruitment after the insertion of the implant: in particular the activation of microglia, astrocytes, and NG2 glia and their migration toward the foreign body. In addition, disrupted blood vessels release inflammatory factors into the parenchyma. C) Chronic immune response to implanted devices. Astrocytes and other glial cells form a physical and chemical barrier around the device. The functionalities of the cells in this region are largely impaired. Glial cell differentiation is reduced and axon remain demyelinated. Reproduced with permission.[155] Copyright 2017, American Chemistry Society.

cascade of biological and biochemical events. In the acute gliotic response to electrodes, both glia and neurons are involved. Typically, after surgical insertion, the electrode implant generates a bidirectional interaction with the tissue, provoking both acute and chronic changes in the patient brain and in turn causing structural degradation linked to the aggressive and reactive cellular environment into which the electrode is embedded. In engineering and material science, these two interactions are usually referred to as biotic and abiotic processes.[129,130] The first includes the cellular response to a foreign body, while the latter refers to impedance increase in time and electrode corrosion. In these processes, microglia and astrocytes play a crucial role in the tissue's reaction, distorting their natural functions and leading to gliosis. This, in turn, triggers dysfunctional behavior in other glial cells, thus altering the normal interactions with adjacent neurons and more generally with the participating neural circuits. The rupture of the blood–brain barrier (BBB) results in an extravasation of serum proteins, such as albumin and fibronectin, together with the infiltration of leukocytes and platelets.[134] This process in turn activates the inflammatory process of microglia and astrocytes. Indeed, microglia start secreting cytokines that, at the beginning, act as proinflammatory biomolecules. These cells lose their normal functions and also trigger nearby neurons to become excitotoxic and neurodegenerative. Then, the microglia start dividing and proliferate, migrating toward and clustering on the implant. The microglial-astrocytes cross-talk activates astrocytes, leading to further increases in the neuroinflammatory cascade. Once activated, reactive astrocytes secrete an abundance of glutamate that causes excitotoxicity.[6] The uncontrolled and persistent glutamate stimulation of neurons might also lead to epileptic seizures and cause neuronal death. Additionally, the impaired capability of astrocytes to control the extracellular environment homeostasis thus creates a harsh environment for tissue regeneration. The result of the gliotic cellular reaction is typically the formation of an encapsulation layer around the implant with a markedly reduced density of functional neuronal cells.[10,135,136]

Given the importance of the topic, immunological and molecular studies characterizing the glial response to implanted devices has been carefully reviewed elsewhere.[10,135] In the next paragraphs, we summarize the main issues, propose alternative solutions and speculate on future trends that will optimize glial interfacing and lead to reduced gliotic reactions.

Despite the unavoidable issues caused by implant insertion, there are several factors that can be tuned to minimize the effects of gliosis, thus prolonging the operability of implants in time. Among these factors, mechanical mismatch between the brain and the device material, specific biofunctionalization of the electrodes and low-impedance nanocoatings can be decisive factors in determining the lifetime of neural interfaces. One of the main issues surrounding interfacing an inorganic material with brain tissue remains the large mechanical mismatch between

these two components. Indeed, several orders of magnitude separate the elastic module of the human brain tissue (10^2–10^3 Pa) and any inorganic material, including silicon (10^{10} Pa) and metals (e.g., platinum, gold, etc.). This large difference triggers a complex interaction after physical insertion of an implant. Examples include penetrating electrodes, where gliosis and disruption of the BBB contributes to an increase of local inflammation, and epicortical devices, where the main issue is friction between the brain tissue and the epidural implant caused by movement of the brain (e.g., periodic pulse due to blood pressure in the vessels). On the one hand, scholars tend to propose organic or hybrid blends to minimize these detrimental effects in an attempt to drastically reduce the mechanical friction between the device and the tissue.[137] However, such soft devices are difficult for surgeons to insert, and they are often thick (several tens of micrometers) to permit the encapsulation of metal tracks. Another possible solution is related to the implementation of ultrathin and ultraflexible devices, where neural interfaces can be integrated together with flexible electronics and deployed onto the dura or below the brain membranes. Usually, devices with a total thickness in the range of few microns can guarantee a certain degree of stretchability and higher level of conformability, even for implants composed of hybrid materials, like polyimide, and active inorganic films, like metals or silicon semiconductors.[138–141] Indeed, thin devices may be a suitable strategy to minimize the impact of the implant on brain tissue. In line with this evidence, in a recent work, Luan et al.[142] demonstrated that ultraflexible nanoelectronic thread electrodes enable glial scar-free implant integration with impedances, noise levels, single-unit recording yields, and signal amplitudes that are stable up to 3.5 months implantation. The reported approach is compatible in vivo two-photon (2P) imaging, and direct contact with the probe did not affect the viability of neurons.

Chemical biomodification of the implant interface by immobilization of bioactive peptides is a strategy that allows for a promising degree of cell selectivity. Indeed, a major advantage in the functionalization strategies of brain implants aimed at directing and controlling glial–interface interactions and/or reducing the gliotic response is the differential and sometimes opposite effect of the functionalized group on neuronal and glial cells. As an example, organosilica sol–gel functionalization[143] by amino end groups has a beneficial impact on neuronal adhesion and neurite outgrowth, whereas the same coating inhibits astrocytic adhesion to the surface. Thus, by amino-bearing, hybrid organo-silica coatings can selectively modulate neuronal cell responses. Similarly, the surface modification of implants with integrin binding peptides might anchor astrocytes to the implants while inhibiting their gliotic hypertrophic proliferation.[143] In principle, selective and spatially distributed biomodification of the implant surface (with peptides, aminogroups, or RGD mimicking motifs) might ensure efficient neuronal coupling to the electrodes, with concomitant efficient and nonadverse interactions of astrocytes with the implants that ensure the stability of the implant and its performance over time.

Other interesting strategies to reduce the inflammatory reaction of glial cells in the brain are the use of antinflammatory agents on inorganic electrodes or the coverage of implants with biomimetic and bioactive coatings to mitigate the neuroimmune inflammatory reaction. Several examples of active biofunctionalization of planar electrodes have been reported, including the implementation of α-Melanocyte-stimulating hormone (α-MSH),[144]—a hormone usually secreted by pituitary cells, astrocytes, monocytes, and keratinocytes that has intrinsic functions inhibiting proinflammatory cytokines—or the local release of Dexamethasone, a synthetic glucocorticoid hormone.[145] Sometimes these biomolecules are directly immobilized on the electrodes, but the usage of nanocoatings or nanoscaffolds strongly enhances their slow release and prolongs the anti-inflammatory action. To this end, dissolvable silk substrates—nanoparticles embedded in bioresorbable polymers and electrospun biodegradable nanofibers—represent valid options to solve the problem.[145–147] Additionally, nanostructured morphology can increase ATP release in astrocytes and downregulate GFAP expression.[148,149] Although these nanocoatings clearly mitigate the inflammatory response of the tissue in the first few weeks after implantation by minimizing the spatial distribution of the glial scarring, the real impact on neuronal function caused by the treatment is still controversial since in many cases the neuronal density remains unchanged. Indeed, the effect may only be temporary and is related to the amount of available drug. Another option for advanced biofunctionalization is cell grafting directly onto the electrodes. In this case, scholars exploit what is known as the "bystander" effect, in which transplanted cells support the host tissue by secreting therapeutic factors, thus promoting healing mechanisms. Although this approach is very promising, large differences are observed between in vitro and in vivo studies. In particular, Wu et al., demonstrated successful culture of hippocampal and cortical neurons on hyaluronic acid-based films fabricated through a layer-by-layer assembly technique, but other authors have not replicated the same results in vivo.[150,151] Purcell et al., demonstrated the beneficial effects of neural stem cells seeded onto Parylene-C probes for at least the first few weeks after implantation. The authors have ascribed the detrimental long-term reaction of the tissue to the degradation of the scaffold containing the cells and to the resulting cellular debris, suggesting ways to improve the design and implementation of future biohybrid devices.[152] A more viable way to obtain a true healing effect that has been proposed is coating the electrode with the biomolecule L1. This molecule has been shown to mediate axon outgrowth, adhesion, fasciculation (including axonal guidance), and neuronal migration and survival, thus promoting cell regeneration.[153] Recent evidence has highlighted the effects of the L1 coating on acute and chronic neuronal and glial responses near neural electrode implants in mice and rats, and from brain, spinal cord to dorsal root ganglion preparations. Furthermore, chronic recording improvement has been demonstrated.[154]

Among strategies aimed at reducing gliotic responses, targeting the expression and function of ion channels, water channels, and other transporters represents a truly attractive and certainly underestimated target for engineering neural implants with reduced gliotic responses. However, this approach has only been exploited in vitro so far.

3.1. Glial Interfaces Based on Polymeric Materials

The use of biograde polymer biomaterials has given some promising results in preclinical animal models: scaffolds are

Table 1. In vitro response of astrocytes to synthetic polymers.

Materials	Type	Technology	Key properties	In vitro response	Reference
Poly(lactic-*co*-glycolic acid)	Nanoparticles	Emulsion	Fast degradation rate (lower than 4 months)	Support of molecular targeting	[182]
Polylactic acid	Porous scaffolds	Thermally induced phase separation	Medium degradation rate (from 4 to 6 months)	Induction of star-like morphology of astrocytes	[181]
	Aligned nanofibers	Electrospinning		Influence on neuroprotective properties of glial cells	[170]
Polycaprolactone	Random sub-microfibers	Electrospinning	Slow degradation rates (higher than 6 months)	High expression levels of glial fibrillary acidic proteins	[169]
Polyethylenglicole	Hydrogel coating	UV light crosslinking	High swelling ratios and good transport properties	Mitigate mechanical damage from micromotion	[183]
Polypyrrole	Conductive coatings	Electrochemical polymerization	Electro-conductive properties	Neural stimulation and recording in vitro and in vivo	[184]

Table 2. In vitro response of astrocytes to natural polymers.

Materials	Cell type	Technology	Key properties	In vitro response	Reference
Collagen Type 1	C8 D1A	Thermal gelation and casting	High biocompatibility Low stability	3D cell–cell interfaces modeling	[194]
Alginate	Primary rat cortical	Ion crosslinking	Good biocompatibility High processability	Increased Reactivity of astrocytes	[204]
High Molecular Weight Hyaluronic Acid Hydrogel	T7–T8 Rat, dorsal hemi-section injury	Hydrogel, chemical synthesis	Good biocompatibility, high hydrophilicity	Reduced astrocyte proliferation,	[194]
Silk fibroin	Primary rat cortical	Films	High biocompatibility, good mechanical properties	Preservation of function al properties, targeted drug delivery	[104,211]

able to deliver and guide cells in the host and to sustain long-term delivery of molecules to the surrounding, damaged gliotic tissue.[138] Among the key features of these scaffolds are polymers that can be properly loaded with ECM proteins, bioactive fragments (i.e., growth factors), or other molecular species, and that possess the right mechanical and surface properties for guiding and controlling the attachment, growth, and differentiation of transplanted cells. Different molecular, polymeric, 2D, and 3D materials have been so far tested as culturing substrates for astrocytes. These studies have highlighted how both chemical and physical factors can play a role in the promotion of adhesion, growth and differentiation of astroglial cells. Mechanical properties such as stiffness, elasticity, and roughness have in recent years received much attention and have proven to be some of the most important parameters regulating cell behavior. Recent studies showed that surface chemistry (i.e., polarity, hydrophilicity, reactive groups) can also affect cell growth and alter the expression of differentiation pathways and other biological functions.[156,157]

In this view, polymeric biomaterials—derived from synthetic or natural origin—have recently been exploited to fabricate "smarter" interface materials (i.e., 3D structures or 2D coatings) that are suitable for reducing the effect of neuro-inflammation and gliosis without influencing probe signal quality and general host response. A collection of the materials recently proposed for interfacing with glial cells, with a particular focus on astrocytes, is described and discussed below (**Table 1**,**2**).

To date, much experimental evidence has confirmed that the biomaterials properties of synthetic polymers, such as stiffness, surface topography, porosity, and molecular transport, play important roles in determining the in vitro responses of astrocytes to the materials.[159] The complexity of the brain environment from which astrocytes and other glial cells originate is mainly due to the myriad of cell–cell and cell–matrix interactions, all of which influence the response to trauma.[160] There is strong interest in the emerging use of biomaterials with highly tunable chemical and physical properties—such as chemically modifiable polymers—that would allow for the alteration of the phenotypes of both transplanted cells and native glia.

In the last five years, several studies have investigated the in vitro response of astrocytes to synthetic polymers and 3D scaffolds (Table 1). Understanding astrocytic adhesion, morphology, proliferation, migration, and gene and protein expression in response to these materials has allowed researchers to synthesize new polymers for the fabrication of 3D scaffolds that may be suitable for interfacing with CNS tissues. Indeed, synthetic polymers allow for the reproduction of the main structural features that are naturally present in the ECM and that are required for cells to adhere and function normally. The need to finely control and optimize production conditions has stimulated significant interest toward the implementation—namely, chemical modification, functionalization—or the revision of currently used polymer-based synthesis and process technologies.

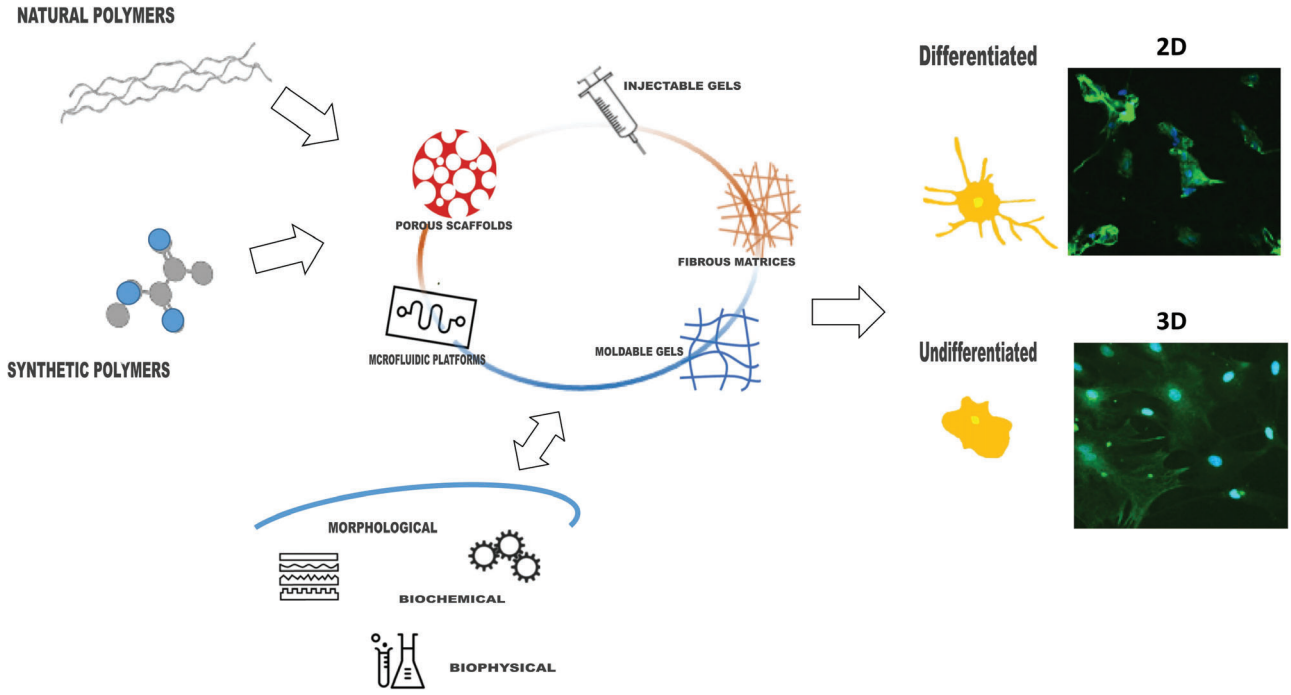

Figure 14. Design of polymeric glial interfaces: polymers from natural source or chemical synthesis can be processed in different forms with peculiar morphological, biochemical, and biophysical properties to mimic the complex 3D microenvironment of brain. In particular, unique topographic signals of fibrous matrices can influence the morphology and astrocyte functions, thus promoting an in vivo-like behavior of cells. Inset) Fluorescent cells reproduced with permission.[158] *Copyright 2020, Elsevier.*

Current technological approaches for the fabrication of brain interfaces are generally based on innovative processes such as electrospinning,[161,162] nanoemulsion,[163] and phase separation, all of which have addressed the need to finely control the polymer structure at micro- or nanometer scale (**Figure 14**). In this context, synthetic polymers with biodegradable or nonbiodegradable properties can successfully be used. For biodegradable polymers, poly (α-hydroxy esters) are often preferred due to their ability to be easily processed and to have the correct levels of mechanical strength and stiffness. Moreover, these materials are well recognized for their biocompatibility (most are FDA approved), nonimmunogenicity, low toxicity, and biodegradability, thus making them suitable for both interface regeneration and molecular targeting. Among them, polyesters of lactic and glycolic acid (PLA or PGA) or their co-polymers (PLGA) can be used in various biological contexts because their biodegradability rate can be controlled by altering the relative PLA:PGA ratios.[164] For instance, the specific degradation mechanism of PLA occurs via nonenzymatic hydrolysis and promotes the formation of lactic acid products that can be easily removed by cellular metabolic processes and that has relevant benefits to the engineered, nanostructured biointerfaces for the CNS.[165] Moreover, the particular chemistry of PLGA copolymers can also guarantee the right transport of therapeutic agents (i.e., antitumor drugs, glial derived neurotrophic factor) across the BBB for efficient targeting of brain regions in the treatment of neurodegenerative diseases, such as Parkinson's Disease.[166]

Alternatively, more chemically stable polymers, such as polycaprolactone (PCL), with long degradation rates (over 1 year in vivo)[167] have been used successfully due to their ability to influence single cytoskeletal protein expression during astrocyte development. Indeed, it is well known that polymeric biomaterials can increase the levels of expression of the cytoskeletal marker GFAP, inducing a more reactive astrocyte phenotype, which may not favorably support regeneration.[168] Accordingly, it was demonstrated that randomly distributed PCL nanofibers, which are fabricated via electrospinning and mimic the specific ECM-like fibrous architecture, can create a less stressful environment for astrocytes, thus resulting in gene expression profiles with biochemical pathways able to regulate proliferation, cell shape, and motility in a way that is more beneficial when compared to bulk films.[169] In line with this evidence, Lau et al. investigated the role of random or aligned organization of PCL fibers on the in vitro response of mouse astrocyte via gene and protein expression studies, confirming the ability of PCL oriented nanofibers to down-regulate GFAP protein expression. Meanwhile, mRNA expression of genes involved in cell motility pathways—namely actin, vinculin and chemokines—neurotrophic factors, antioxidants (glutathione S-transferase α1) and the glutamate transporter EAAT2 was upregulated when astrocytes were cultured on PCL random nanofibers.[170] Several studies have demonstrated that the topographic and morphological features of PCL-based electrospun fibers are able to drive astrocytic adhesion and survival over the long term (**Figure 15**).

In addition, Saracino et al.[158] demonstrated that aligned PCL-based fibers induced a dramatic rearrangement of the actin-cytoskeleton as well as focal adhesion point number and spatial distribution in astrocytes. Interestingly, structural changes observed in elongated astrocytes are not correlated with alterations in their electrophysiological properties, i.e., potassium

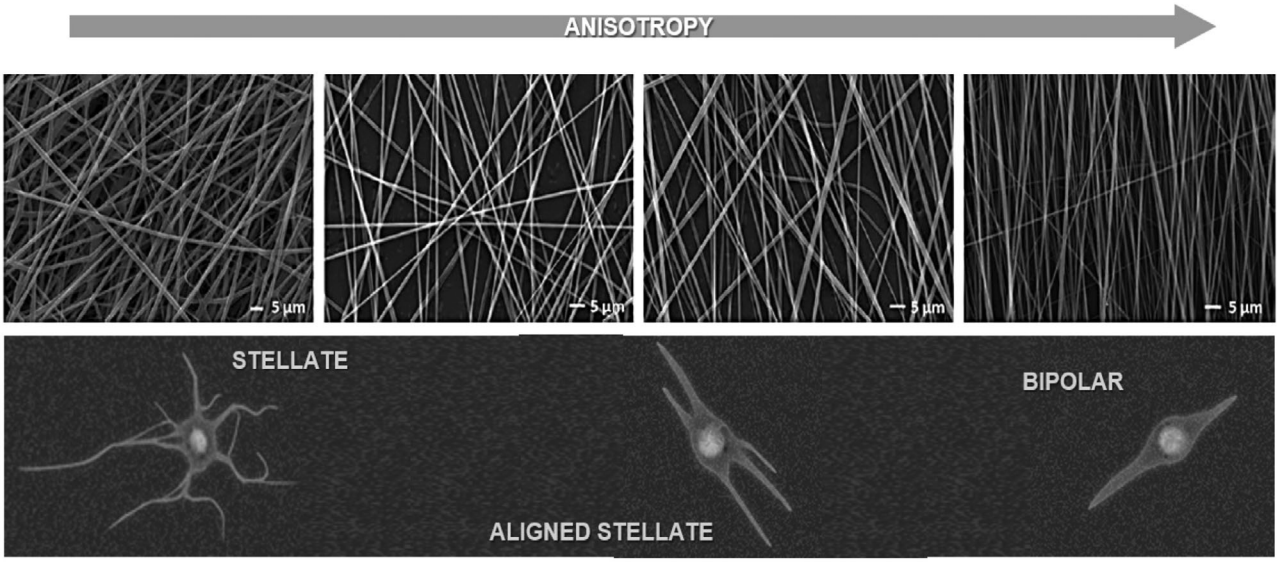

Figure 15. Surface anisotropy related to the astrocytic response ton 3D culture: Effect of fibers alignment and anisotropy on the morphology of cell bodies. On the top, PCL-based fibers fabricated via electrospinning with different anisotropy degree were reported. Reproduced with permission.[171] Copyright 2014, Springer Nature.

channel,[158] thus suggesting that PCL electrospun fibers are permissive substrates able to drive specific properties in astrocytes depending on the target applications.[172] Moreover, PCL can be chemically modified during synthesis to include different copolymers with properties suitable for the CNS. For instance, Pires et al. demonstrated that electrospun fibers made of poly(trimethylene carbonate-*co*-ϵ-caprolactone) (P(TMC–CL))—a copolymer with high caprolactone (CL) content including grafted TMC—are readily able to promote axonal growth, overcome myelin inhibition, and influence microglial cells during CNS regeneration.[173,174] Hence, in agreement with previous experimental evidence on similar polymeric substrates (i.e., poly methyl methacrylate),[175] these studies confirm a strict correlation between astroglial cell behavior and specific topographic signals, due to the fiber alignment.

As for nondegradable polymers, soft hydrogels have been preferentially used as coatings to modulate glial scar formation around neural implants. The hydrogels accomplish this by reducing small strain between the implanted electrodes and the surrounding brain tissue that occur due to local micromotions. For instance, PEG–dimethacrylate (DMA) coatings with controlled thicknesses (25–100 µm) and with elastic moduli similar to those of brain tissue (close to 5 kPa)[176] were optimized to mitigate mechanical damage from the micromotion caused by commercial neural implants. PEG is universally recognized as biocompatible for brain cells[177] and shows specific physical properties that can be tailored by modification of the chemical synthesis process in order to match the necessary properties of brain tissue[178] Additionally, the functionalization via surface modification techniques also allows for the synthesis of a series of newly-modified biomaterials (i.e., arginine- and polyethylene glycol-modified polymers) with enhanced fluid transport properties that are able to improve the interface between the implant and primary cultured human neural cells (neurons and astrocytes) and that support neurotrophic factor expression and gene targeting in mice.[179]

Wissel et al.[180] investigated the growth of glial cells on ultrathin poly(*N*,*N*-dimethylacrylamide) (PDMAA), poly(2-ethyloxazoline) (PEtOx), and poly([2-methacryloyloxy)ethyl] trimethylammoniumchlorid) (PMTA) films grafted onto a glass slide via photoreactive treatments. They demonstrated that glial cells attached only to the PMTA films. As PMTA is the only polymer with a cationic charge, the authors assumed that this charge improves the adhesion of glial cells to the material.

A noteworthy and key problem related to glial scar formation is the local increase of tissue impedance, which is related to a reduction in the portion of the interfaced tissue effectively activated by electrical stimulation (up to 50%). In this view, recent discoveries of polymers with electrical properties (i.e., polythiophenes, polyanilines, etc.) have suggested that designing electroactive biointerfaces will support neurite guidance for neuroregeneration.[185] The particular properties of these polymers, namely electroconductivity, can effectively support the functional activities of cells at the device interface both in vitro and in vivo. This cellular support occurs directly by electrical stimulation,[186] indirectly by the release of neurotrophic factors, or by the local interaction with decorating molecules on the surface of the implant.[187] Regardless of the method of support, all of these technologies greatly contribute to the development of innovative nerve disease therapies.

3.2. Glial Interfaces Based on Natural Polymers

Natural polymers have generated much interest for potential use in clinical applications targeting astrocyte structure and function in neurology (Table 2). Indeed, they can efficiently work as a) local carriers able to target molecular signals directly to the

injury site,[188] and b) as scaffolds able to offer the required structural support for cell attachment, proper nutrient and oxygen supply, and protection to host cells, thus promoting the ex novo formation of the extracellular matter.[189] These abilities are associated with their recognized biocompatibility and specific hydrophilic properties that allow for the formation of 3D networks with tunable physical and chemical properties that might allow for the retainment of up to 90% w/w water.[190] Natural polymers have been extensively used in brain surgery, thanks to the use of injectable formulations based on Matrigel or that include hyaluronic acid derivatives, collagenous proteins, polysaccharides, or self-assembling peptides and proteins. As a function of these properties, they can be engineered and synthesized in specific ways—in order to play different roles in terms of matrix analogues, functional agents, or molecular carriers—and morphologically adjusted to fit complex physiological geometry for the CNS.[191] For example, hyaluronic acid-based hydrogels with different molecular weights or grafted with different peptides (i.e., RGD, YIGSR, IKVAV, and RDG adhesive peptides) have shown varying degrees of stiffness, as a function of the chemical modification, thus underlining a correlation between mechanical behavior of the materials and the biological response of human-induced pluripotent stem cell-derived neural progenitor (hiPS–NPCs) in terms of spreading and cell attachment.[192] In agreement with other studies,[193] these results confirmed the idea that the mechanical similarity of soft hydrogels may contribute to the support of the biological functions of native brain tissue.

Several works have demonstrated that the biological properties of Collagen I—, i.e., the more diffused ECM proteins in the adult nervous system at the level of basement membranes of the BBB and in the neuromuscular junctions—play a relevant role in CNS development and in 3D remodeling of brain tissue during regeneration.[194] Importantly, collagen was officially declared a neuro-compatible material, and its implementation was also addressed in the development of several commercial products for in vivo applications. It follows that collagen has been widely used as substrate to treat the injured CNS[195] as well as a scaffold material for cellular therapy.[196] It is bioresorbable and can be easily incorporated by host tissue. However, collagen-derived matrices, such as Matrigel, provide a broad range of factors suitable to support cell functions with some limitations for isolating and discerning the cell signaling mechanisms due to the inherent complexity and variability of the biomatrix properties.[197] Moreover, different formulations of collagen materials were successfully investigated in recent years to study specific responses of cells (i.e., progenitor cells, glial cells, astrocytes). For example, collagen was combined with other biopolymers, such as HA, to study the in vivo response of mouse embryonic neural progenitor cells (NPCs) cultured in HA-heparin-collagen and transplanted into a murine model. This study demonstrated that collagen-based scaffolds swell slightly into the implant site, protecting graft cells from microglia and macrophages and increasing survival of NPCs, thus suggesting an interesting in vivo compatible scaffold for the brain.[198] Astrocytes themselves have been shown to release collagen in different forms (Type I, III, and IV), both under physiological conditions and also during glial scarring. Different bioactive agents have also been grafted to collagen, including macromolecules to support neuronal outgrowth.[174] Along this line, Hlady et al.[201] recently investigated the growth and response of astrocytes cultured in vitro on collagen gels with surfaces patterned and embedded with human plasma fibrinogen (FBG) and three extracellular matrix components, namely aggrecan (AGG), FBG, and laminin (LN). Differently from pure collagen substrate, the protein stripes were able to align primary astrocytes and to ensure their viability for 4 days in vitro, thus indicating negligible toxicity in the short term. Moreover, when aligned on AGG, FBG, and LN proteins, astrocytes showed reduced chondroitin sulphate proteoglycan (CSPG).[201]

Alternatively, ionic sensitive polysaccharides, such as alginates, were also considered attractive for several reasons, including a) mechanical properties that recapitulate those of the brain[200] and b) inert backbone structure that allows for control of the scaffold stability with benefits in terms of recovering cells for further biochemical and cellular assays.[199,201] These particular properties allow for the recreation of the glial scar environment by stimulating astrocytes and widely changing polymer features by tuning their physical properties via physical or chemical crosslinking methods.[202] For instance, the response of cerebral astrocytes to 3D alginate scaffolds with different mechanical properties was tested in the presence of meningeal fibroblasts, thus mimicking the stimuli related to fibroblast infiltration occurring after CNS injury.[203] In agreement with previous studies, mechanical properties of alginate also play a role in the formation of new CNS tissue and glial scar formation,[204] influencing mechano-transduction processes and, ultimately, astrocyte reactivity and both ECM production and composition in the surrounding environment.

Among natural polymers, silk fibroin (SF) obtained by *Bombyx mori* cocoon, is an attractive material for consideration as a glial interface.[205–207] In light of its excellent biocompatibility, tunable biodegradability, and suitable functional properties (i.e., mechanical, optical, dielectric),[206–208] SF-based materials have been extensively investigated for neural tissue engineering and bioactive molecular release.[206–208] Notably, through a water-based and sustainable reverse engineering process,[209] an aqueous-based SF solution can be obtained from the cocoon fiber, called regenerated silk fibroin (RSF). RSF can be processed in various forms (i.e., films, gels, fibers, porous scaffolds, and sponges) with defined chemophysical properties (i.e., defined thickness, mechanical properties, time-controlled biodegradation). Moreover, RSF can be blended, or the silk substrates can be functionalized by means of different chemical functionalization approaches to obtain almost indefinite amount of silk substrates with multiple pharmacological, photonic, electrical, and optical properties.[206–208] The flexibility of silk thin films has been exploited on ultrathin and conformable electrodes aiming to match the mechanical factors needed for reducing the gliotic response.[210] All these remarkable features make silk substrates unique as a glial interface or as a device-integrated component due to the ability to interact with and control neuronal cell function. Accordingly, our group pioneered the use of SF as a permissive interface for the growth of primary rat neocortical astroglial cells. SF films allow survival of primary astrocytes up to 3 weeks in vitro, without detrimental effects on GFAP and without inducing expression of the gliotic phenotype.[211] Highlighting the importance of monitoring functional properties, the work showed that the electrophysiological properties of astrocytes are not significantly different when they are grown on SF compared to when

they are grown on standard Poly-*D*-Lysine coated coverslips.[211] Nonetheless, SF can be an ideal vehicle for the delivery of trophic compounds, such as the purine guanosine, to astrocytes and thus induce a large inward rectifying potassium conductance and parallel increase of Kir4.1 protein channel expression in vitro.

The reactivity of astrocytes induced by silk fibroin has been investigated in vivo.[207,110] Notably, silk fibroin has been integrated into organic optoelectronic polymeric devices based on P3HT/PCBM.[110,212] When used as a retinal prosthesis, it was shown that the implanted device induced less gliosis and reactivity in retinal Müller glia. Moreover, the integration of silk fibroin as a dielectric[208] was demonstrated in the structure of organic field effect transistors, capable of stimulating neurons, and astrocytes.[114,115] These findings validate the use of silk as an effective glial interface for future multifunctional investigations of astrocytes.

Processing methods can critically influence the chemophysical, structural and surface properties of SF films and substrates. Changes in the conformation, biodegradation rate, and texture of silk films, mostly affecting the surface properties of the films, can modulate and tune the astrocytic adhesion and growth. Interestingly, primary astrocytes and primary neurons display different responses, pointing to cell specific molecular pathway activations as a result of the interactions between the neural cell and the material interface. Particularly, several studies have demonstrated that hydrophobic properties contribute to the support of neuron attachment and astrocyte proliferation, whereas hydrophilic ones mainly promote neurite outgrowth.[104,213]

4. Conclusions and Future Trends

It has become evident that astrocytes, once considered merely the glue of the brain, exert a key role especially for those aspects regarding physiology and pathology of the human brain.[215] Indeed, astrocytes are connected not only to neurons, but they form complex networks with each other by using specialized channels that allow the diffusion of nutrients and ions into this intricate structure, thus amplifying the range and scale of synaptic regulation induced on neurons.[216] According to the reported studies, the brain can be considered a tangle of mixed networks of astrocytes and neurons, where i) astrocytes represent a further key element integrated into the neural network; ii) besides the electrical excitability of neurons, astrocytes exhibit a specific excitability based on calcium signaling; iii) considering the complexity of the neural network, astrocyte–astrocyte connectivity and astrocyte–neuron connectivity have to be taken into account together with the standard neuron–neuron connectivity; iv) network functionalities of the brain are mediated not only by neurotransmission but also by gliotransmission. Thus, the communication between and inside these networks plays a crucial role to understand the fundamental mechanisms occurring at different temporal and spatial scales that affect brain normal activity, its plasticity as well as pathological dysfunctions.[217,218]

Over the past few years, the progress in biomaterial science, engineering, and bioelectronics have allowed for the advancement of several technological breakthroughs, offering unique opportunities to scientists interested in the "other brain"[218] to address new challenging biological questions. We selected a broad number of examples to illustrate how novel devices and interfaces can be instrumental in tackling specific questions related to glia and specifically to astrocytes' functional, structural, and biological mechanisms. We propose glial engineering, glial interfaces, and gliophotonics as emerging fields that complement neural engineering and neuronal targeting approaches (**Figure 16**).

The advantages and pitfalls of using the selected materials, interfaces and devices were thoroughly discussed. It appears obvious that astrocytes can sense material surface properties since mechanical features, electrostatic forces, and hydrophobic:hydrophilic ratios have prominent roles in glial interfacing in vitro. It is plausible that the increased surface area of nanostructured probes offers multiple possible sites for interactions between astrocyte leaflets and the nanostructured surface, ultimately favoring astrocytic process outgrowth and differentiation in vitro. The molecular mechanisms behind these observations, though, are still unclear. Evidence from our labs suggests that the capability of astrocytes to sense biomaterials and nanostructured interfaces is a triggering mechanism for cell volume regulation, such as water and ion transport, as well as for actin cytoskeleton rearrangement (Mola, Saracino et al., unpublished). The latter mechanism would suggest that astrocytes respond to increased mechano/surface stimuli, implicating adaptive local rearrangements of cellular volume, which is known to be essential and necessary for the outgrowth and differentiation processes.[13,220,221]

Regarding the ability of astrocytes to respond directly to electric and photonic stimuli, the literature is still limited and open to new insights. On the other hand, photonic sensing and monitoring of astrocytes and glia by two-photon imaging or, more recently, by high resolution microscopy is already an established practice in glioscience. Even though magnetic resonance imaging of glial cells is already in place in the clinics, broader applicability would be achieved by developing tools that enable specific sensitivity for different glial cells—specific to cell type—or for specific tools for sensing their functional state. In this venue, hyperspectral imaging techniques to uncover the signature of gliopathologies are currently being investigated for pain research.[222]

It is worth noting that the permeability of the astrocytic membrane to ions and water and the changes to calcium signaling are accompanied by structural changes to the actin cytoskeleton and to GFAP; these modifications are common markers for the astrocytic response to pharmaceutical drugs, acoustic waves,[223] electromagnetic fields, light, mechanical forces, or altered temperatures that are caused by nanobiomaterial interfaces, electrical stimulation, photonic stimuli, etc. It follows that astrocytes use adaptive mechanisms that are known to be essential for their homeostatic function in vivo and that are lost during brain dysfunction.

Given the need to rescue structural, functional, and morphological features of astrocytes after injuries, targeting the molecular and physiological expression of potassium channels (i.e., Kir4.1), water channels (i.e., AQP4), calcium sensors (i.e., TRPV4 and TRPA1) as well as their structural correlation with the actin-cytoskeleton and with adhesion proteins (such as Connexin, Pannexins, GlialCam) could represent a revolutionary clinical approach. When combined with biomaterial and device strategies for designing therapies and advanced diagnostics, it may finally be possible to study and treat complex brain disorders

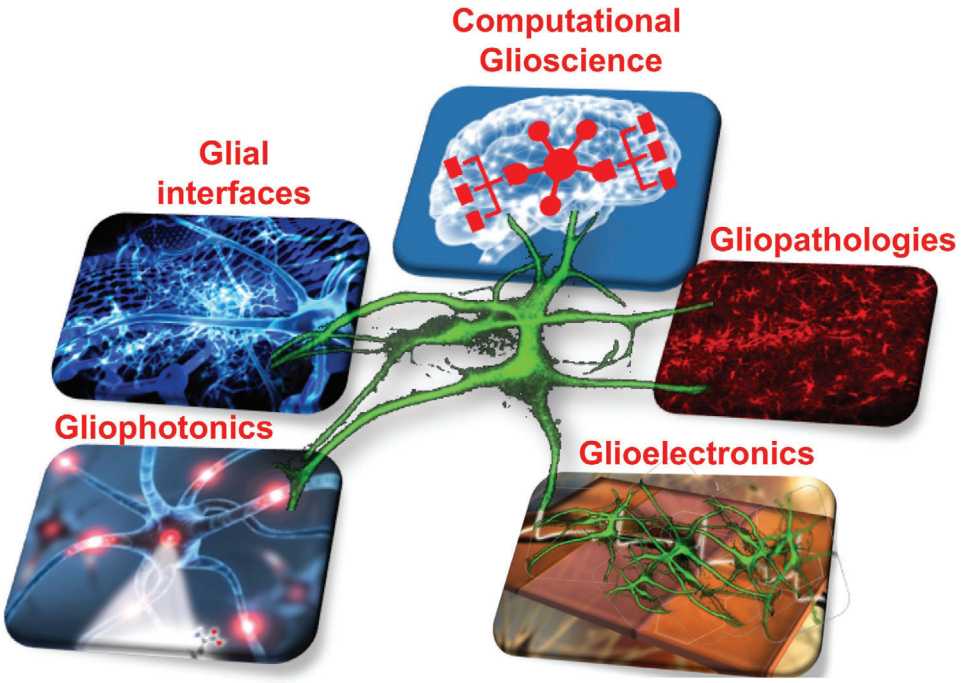

Figure 16. Emerging scientific fields surrounding astrocytic structure and function. Glial interfaces and glial electronics will provide multiscale biomaterials and electronic devices to stimulate and record astrocytes dynamics. Computational Glioscience is devoted to computing and predicting the role of astrocytic dynamics in brain function and dysfunction. Gliophotonics, still at its infancy, is now mainly confined to optogenetic and chemogenetic methods. Moreover, studies using organic optoelectronics and infrared photostimulation are very promising. Insets) Gial Interfaces is adapted with permission.[124] Copyright 2018, The Royal Society of Chemistry. Gliophotonics is adapted with permission.[219] Copyright 2014, Wiley-VCH GmbH.

characterized by loss of homeostatic properties of astrocytes (epilepsy among the others), brain edema (ischemia, ictus), or neurodegenerative diseases (Alzheimer's Disease, Retinitis Pigmentosa, Neuromyelitis Optica).

Besides the need for astrocyte targeted therapy, glial engineering can offer unprecedented insights into the molecular and cellular dynamics that make up astrocytes' roles in cognitive function. The active participation of astrocytes in synaptic transmission is now considered an established fact that can no longer be ignored.

Brain machine interfaces and implanted devices allowing for bidirectional communication (stimulation and recording) with the brain have so far been considered for targeting neurons. However, Transcranial direct brain stimulation, which has been proposed to improve human performance, has a major effect on astrocytic calcium signaling in rodents in vivo. Nonetheless, studies in vitro demonstrate the selective ability of astrocytes to respond to different stimulation protocols compared to neurons.[114] Finally, the tradition of considering astrocytes to be nonexcitable cells—displaying only passive currents—will likely be disproved by nanoelectrophysiological recording tools, which have already shown that in vitro astrocytes have the ability to generate local small and slow membrane voltage oscillations in the low frequency range.

We would not be surprised if these continued observations can be translated into designs for electrical/photonic/ultrasound/electromagnetic devices aimed at improving or rescuing the brain's cognitive function with a BCI targeting both astrocytes and neurons.

Finally, a major interesting issue not described here is the need for a bioinformatics toolbox that might analyze, reflect, predict and—in the long-term—personalize the neuroglial cell interactions. The so-called computational glioscience is a challenging and important gliodiscipline that has received growing interest from scientists and stakeholders because of its possibility to redefine reinforcement learning or machine learning approaches.[224]

In conclusion, it is evident that, although 150 years have passed since their discovery, astrocytes, and the glioscience field are just the tip of the iceberg. Despite the plethora of evidence supporting the fact that astrocytes modulate local neural circuits, networks, and complex behaviors, the fields of neuroscience together with chemistry, engineering, and materials science is still dominated by an interest in neurons rather than in glial cells.

Equally importantly, wide multidisciplinary efforts are needed to transfer existing and new knowledge among glioscience and biomaterials science, engineering, chemistry, device technology, and photonics to focus on targeting glial cells in addition to neurons. Doing so will bring global attention to the accumulated knowledge on glial cells, glial interfacing, and glial engineering as fields ripe for the investment of research and innovation that will greatly benefit brain science and neurology.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

L.M. contributed to the concept of glial engineering, to manuscript organization, wrote, and edited the manuscript. V.G. wrote the section on polymeric glial interfaces, Ma.M., A.C., and E.S. contributed to the manuscript writing and editing. L.A., M.M., and R.Z. contributed to the discussion and to the editing of the manuscript. V.B. coordinated the effort, developed the concept of glial interfaces and glial engineering, wrote and edited the manuscript.

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