Chapter 8
Therapeutic Approaches for Stroke: A Biomaterials Perspective

Artur Filipe Rodrigues, Catarina Rebelo, Tiago Reis, João André Sousa, Sónia L. C. Pinho, João Sargento-Freitas, João Peça, and Lino Ferreira

Abstract Stroke is a leading cause of death worldwide and poses significant societal and healthcare challenges due to functional impairment of the brain. In order to fully restore brain function, innovative approaches have aimed to regenerate the injured tissue and to restore neuronal circuitry. In the last 5 years, stem cells have been consistently explored in clinical trials for tissue regeneration. Recent technological progress regarding the use of stem cell-derived extracellular vesicles has also shown promise toward the administration of cell-based therapies exploiting paracrine signaling. In addition, neuromodulation using different stimulation modalities has become increasingly investigated in the clinic as a non-invasive strategy to promote functional recovery. This approach contrasts with invasive strategies using devices capable of delivering electrical pulses in deep regions of the brain, which nonetheless are well-established in the clinic for the treatment of other neurological disorders. This chapter reviews the latest approaches covering brain tissue regeneration and neuromodulation, and discusses their limitations for clinical translation. Preclinical investigations on the use of light for neuromodulation in optogenetics have sparked the development of biocompatible interfaces capable of coupling optical stimulation with electrical recording. These biointerfaces require novel materials whose physicochemical properties are discussed herein.

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8.1 Introduction

Stroke is the second leading cause of death worldwide and it is characterized by neurological impairment caused by vascular failure, which deprives focal areas of the central nervous system (CNS) from oxygen and nutrients supplied via the bloodstream (Fig. 8.1) [1]. Stroke encompasses clinical events, primarily occurring in arteries, which are triggered by different vascular pathologies: ischemic stroke, intracerebral hemorrhage, subarachnoid hemorrhage, and cerebral venous thrombosis [2]. On the one hand, ischemic stroke is the clinical consequence of local obstructions in the brain vasculature by blood clots, resulting in extensive cell death in the ischemic area [1]. On the other hand, hemorrhagic stroke results from a rupture of a weakened intracranial blood vessel, which can be caused by high blood pressure, amyloid angiopathy, coagulopathies, or a structural blood vessel abnormality (e.g. aneurysm, arteriovenous malformation, neoplasm) [2]. These etiological features underlying hemorrhagic stroke require immediate action to control blood pressure and, in certain cases, the administration of procoagulant agents and/or surgery to drain intracranial blood. In contrast, the most effective strategy to treat ischemic stroke is simply removing the blockage to the blood flow, either by intravenously administered drugs or endovascular mechanical therapy [2, 3]. Compared to hemorrhagic stroke, the variety of treatments for ischemic stroke has increased the chances of survival by 5-fold, saving every year the lives of around 80 million people worldwide [1]. However, current clinical practice has not evolved in the management of long-term associated morbidities [4]. It has primarily focused on the patients' behavioral changes to prevent relapses by adopting correct occupational habits such as a poor diet, physical inactivity, and smoking. These have been associated with metabolic and cardiovascular risk factors including high blood pressure and cholesterol levels in the blood, as well as cardiac arrhythmia and diabetes [1]. In addition, focused physical therapy has enabled functional rehabilitation of muscle movement and mobility, albeit with limited recovery, especially from other common impairments such as speech, language, vision, swallowing, and cognition [5].

Although these efforts have reconfigured neuronal networks disrupted by extensive brain damage, they are insufficient to fully restore function. In this context, biomaterials have assisted the development of advanced therapies such as electrical stimulation and cell-based therapies, which have been employed to remodel neural circuitry and to trigger regeneration of the affected brain tissue. The present chapter describes the existing state-of-the-art for the treatment of stroke and some of the most recent innovations in cell-based therapies and neuromodulation using light and electricity, whose combination is anticipated to be of clinical relevance in the near future. Stroke therapies have mostly relied on non-invasive strategies such

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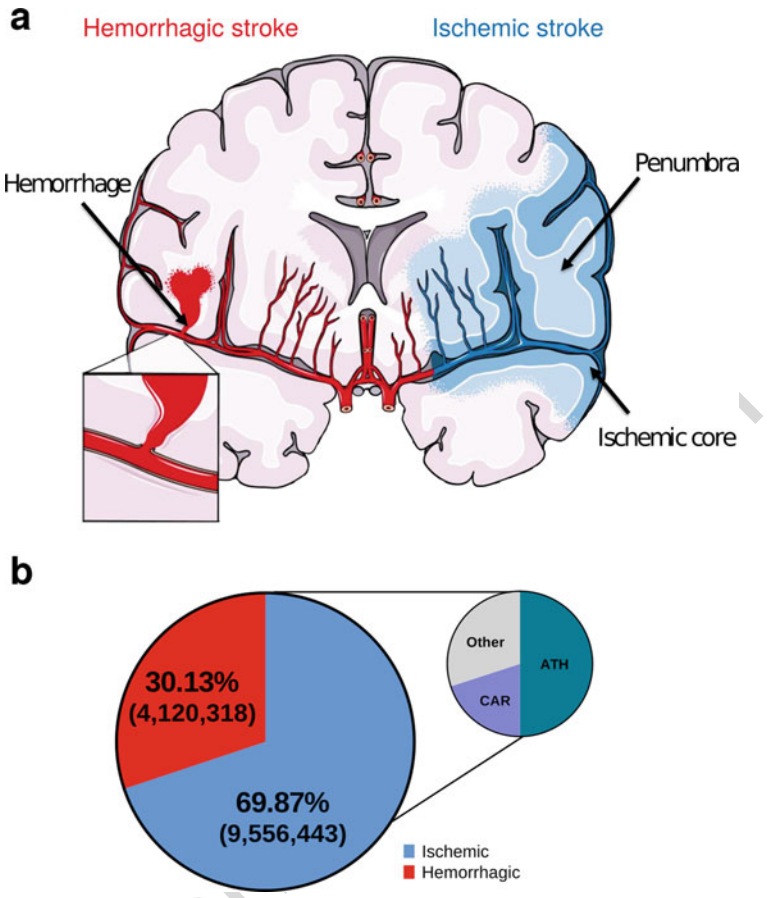

Fig. 8.1 Stroke etiology and prevalence. (**a**) Schematic representation of the main stroke subtypes, which can be classified by the deprivation of brain regions from access to oxygen and nutrients due to either the disruption (hemorrhagic) or occlusion (ischemic) of blood vessels. Adapted from images from Servier Medical Art by Servier (http://smart.servier.com), licensed under a Creative Commons Attribution 3.0 Unported License. (**b**) Although the overall number of stroke events has decreased in recent years, more than 13 million events were registered in 2016. Hemorrhagic stroke was less frequent than ischemic stroke, and it can be characterized by its onset in the brain or the subarachnoid space [1]. Contrarily, ischemic stroke is most frequently triggered by the rupture of atherosclerotic plaques from major vessels (i.e. large-vessel atherosclerosis, ATH) [6, 7]. Another frequent subtype of ischemic stroke is cardioembolism, which consists of the release of blood clots or atherosclerotic plaques accumulating in the cardiac tissue. Other ischemic stroke subtypes include small vessel occlusion triggered in patients suffering from hypertension or diabetes and rare events caused by non-atherosclerotic pathologies or other unknown factors [6, 7]

as transcranial stimulation of the brain and the administration of medicines to minimize tissue damage. Invasive neuromodulation techniques such as deep brain stimulation are highly effective in remodeling neural circuitry; albeit still generate long-term complications resulting from poor device biocompatibility. We propose the development of novel devices with biodegradable materials and minimally invasive implantation strategies to expand the therapeutic possibilities for stroke. The use of biomaterials to modulate cell activity will be discussed, with particular emphasis on material properties leading to improved biocompatibility and electrical conductivity.

8.2 Therapeutic Approaches to Stroke

8.2.1 Stroke Epidemiology and Pathophysiology

A variety of etiological mechanisms may trigger an ischemic stroke. The TOAST study has classified ischemic stroke based on the following causes: large artery atherosclerosis, cardioembolism, small vessel occlusion, stroke of other determined etiology, and stroke of undetermined etiology [6, 7]. Knowledge of these mechanisms for each patient is crucial to adjust secondary prevention with tailored therapies. Clinically, there are some noticeable symptoms associated with stroke, ranging from a minor central facial palsy to an acute coma. Other symptoms include numbness in one side of the body, difficulty understanding other people, difficulty in seeing with one or both eyes, gait problems and discoordination, dizziness/vertigo, and severe headache. These symptoms correspond to a cerebral loss of function of sudden onset, whose severity depends on the anatomy of the occluded/ruptured artery and collateral systems, as well as the patient's age and gender, and the presence of comorbidities [8]. The common triad of face drooping, arm/leg weakness, and speech difficulties (FAST acronym) should warrant an immediate call for help through pre-hospital emergency systems, as response time is critical at this stage [9]. Indeed, determining etiology and location of the infarct and rapidly restoring an adequate systemic blood pressure and irrigation will dictate the final infarct size and subsequent neurological consequences [8, 10].

Histologically, stroke is characterized by an ischemic core surrounded by a "penumbra" region, which can be monitored using non-invasive imaging techniques such as computerized tomography (CT) or magnetic resonance imaging (MRI) [2, 7]. Although imaging tools are a valuable asset to identify anatomical regions that are damaged during and after stroke, the quality of patient recovery requires specific functional predictors to guide rehabilitation. Clinical management of stroke has relied on biomarkers for the molecular processes taking place in the brain, including inflammation, hemostasis, and cell death [11]. At the ischemic core, where blood flow is most severely restricted, excitotoxic and necrotic cell death occurs within minutes due to oxygen and glucose deprivation, which causes glutamate

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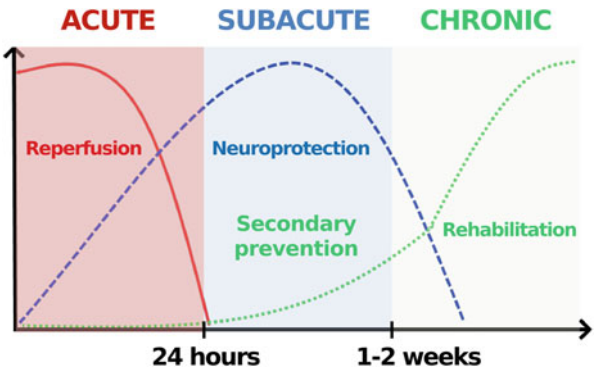

Fig. 8.2 Ischemic stroke management over time. During an ischemic stroke event, the initial priority is to rapidly irrigate the brain tissue deprived from blood circulation. In the following days, oxidative stress and extensive cell death are mitigated by the administration of neuroprotective agents. Continuous monitoring of brain activity is required to prevent secondary stroke events. Finally, long-term rehabilitation and physiotherapy aims to restore brain functions

release and mitochondrial dysfunction [12]. Activation of apoptosis, necrosis, and autophagy pathways disrupt the blood–brain barrier (BBB) and trigger peripheral immune responses to the lesion site, which further enhance oxidative degradation of several biomolecules, such as proteins, lipids, and DNA. As a result, cell death is progressive toward the penumbra, where collateral blood flow can buffer the effects of tissue damage at the ischemic core [13]. Although elevated serum cytokine levels and increased production of inflammatory mediators in circulating and splenic immune cells can be detected within hours after ischemia [14], there are currently no specific biomarkers to detect brain damage [11].

8.2.2 Clinical Standard of Care

The management of an ischemic stroke is multiphasic and time-bound (Fig. 8.2). First, an acute/early stage prioritizes the reperfusion of the occluded artery, followed by a subacute stage where monitoring, prevention of stroke complications, preservation of the surviving brain, and etiologic investigation take place. Finally, a chronic stage focuses on rehabilitation and prevention of secondary stroke events.

Current treatment options in the acute phase, although with a relevant improvement on the clinical outcome, have a limited time window to be applied. Stroke patients may be subjected either to pharmacological treatment for the dissolution of blood clots in ischemic strokes and/or to mechanical removal of the clot by endovascular procedures [15]. Tissue plasminogen activator (tPA) is the only thrombolytic drug that has been clinically approved by both the Food and Drug Administration of the USA (FDA) and the European Medicines Agency (EMA).

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Patients treated with tPA are at least 30% more likely to have minimal or no disability 3 months after stroke [16]. However, treatment time is crucial for this outcome. No significant improvements were observed when tPA was administered more than 4.5 h after symptoms onset [17, 18]. Systemic delivery of tPA promotes the conversion of plasminogen to plasmin, which will bind and degrade fibrin, dissolving blood clots. Efficacy of tPA treatment can be extended up to 24 h after the development of symptoms by mechanically destroying the blood clot [19]. Thrombectomy is a catheter-based image-guided intervention for the mechanical removal of blood clots in large vessels through aspiration or stent-retrieval. This procedure showed remarkable improvement in the recovery of neurological function of patients suffering from large-vessel occlusion [20]. Nonetheless, patient selection and timely reperfusion are crucial for a successful outcome. Only 13%–20% of total acute ischemic stroke patients are eligible for endovascular therapy [21], due to factors such as patient's age, stroke severity, and anatomical location of the occlusion, as well as the history of previous disability/dependence episodes [22].

The aforementioned pharmacological and mechanical therapies rely on the re-implementation of blood flow to stop the onset of tissue damage. In contrast, adjuvant neuroprotective treatments attempt to minimize the signaling pathways that are subsequently activated after loss of blood flow and lead to neuronal death [23]. Currently, there are no approved pharmacological treatments with neuroprotective effects [15]. Nevertheless, several agents have been studied and are under development, particularly now that restoring blood flow to the occluded artery has become clinically established [24]. The aimed neuroprotective strategies are focused on addressing excitotoxicity, i.e. cell death associated with an excess of excitatory neurotransmitters [25], immune and inflammatory responses [26], and apoptosis [27]. Among these, statins are a main group of neuroprotective agents that act inhibiting hydroxylmethylglutaryl coenzyme A reductase, which cause a reduction in low-density lipoprotein (LDL) cholesterol levels. In addition to this anti-thrombotic effect, statins seem to have other roles in the treatment of the pathophysiology of ischemic stroke [28], which have been investigated in clinical trials [29, 30].

Altogether, clinical management of stroke requires comprehensive hospital units with multidisciplinary teams dedicated to mitigate permanent neurological disabilities which, if unrecovered, pose a huge burden to society [31, 32]. However, this strategy has not been fully successful. Recently, there is a shift toward innovative neurorestorative treatments focused on restoring brain tissue and improving neurological function after damage. They aim to solve some of the aforementioned caveats, including the short time window for therapy and the inclusion of patients that were otherwise excluded from a therapeutic solution.

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8.3 Advanced Therapies for Stroke

8.3.1 Cell-Based Therapies

Due to the limitations of conventional therapies and innovative adjuvant approaches, regenerative medicine has emerged with the aim of restoring brain function in a post-acute stage of stroke. The generation of neurons in some parts of the adult mammalian brain (e.g. the subgranular zone of the hippocampal dentate gyrus and the subventricular zone [SVZ] located outside of the lateral ventricles) provides a possible therapeutic solution for restoring neural function. However, this is still a debated topic following recent evidence with apparently contradicting outcomes [33, 34]. In fact, endogenous repair mechanisms including neurogenesis, synaptogenesis, glial cell activation, and angiogenesis are triggered after ischemic stroke [35]. Nevertheless, if any novel neurons are generated, they are not enough to repopulate the injured site. In addition, angiogenesis is compromised in older patients [34], which poses additional barriers to the restoration of lost neural circuitries [36]. Cell-based therapies are therefore positioned to potentiate endogenous mechanisms and overcome pathophysiological boundaries set by ischemic stroke. Two conceptually different approaches for regenerative therapy after stroke involve cell transplantation and cell recruitment (Fig. 8.3a).

8.3.1.1 Cell Transplantation

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It implies the use of stem/progenitor cells that can be originated from the patient itself (autologous) or from donors that are genetically similar (allogenic) or identical (syngeneic). These cells can be derived from either fetal tissues (e.g. umbilical cord and placenta) or adult tissues (e.g. bone marrow, adipose tissue, olfactory mucosa, and dental pulp) and have been tested over the last 10 years for the treatment of ischemic stroke in the clinic [40]. The most advanced technology consists of the extraction of multipotent adult progenitor cells from the bone marrow of healthy donors (e.g. MultiStem® from Athersys). An exploratory Phase II clinical trial with MultiStem® pointed a favorable clinical outcome for patients that received a single dose of the product 24–48 h after the occurrence of the stroke [41]. The MASTERS-2 Phase III trial to employ MultiStem® as an "off-shelf" product for stroke treatment is now underway [42].

With such a variety of cells according to their source and tissue origin, a main challenge toward clinically relevant cell therapies is to generate high amounts of the optimal cell type. Neural stem cells (NSCs) have the capacity to differentiate into neurons, astrocytes, and oligodendrocytes, what makes them good candidates for effective transplantation and attenuation of the cell loss associated with ischemic stroke. Mesenchymal stem cells (MSCs) have been also investigated to arrest stroke-associated cell death [43]. Compared to NSCs, MSCs can be readily isolated from non-invasive tissue sources such as dental tissue and amplified ex vivo for

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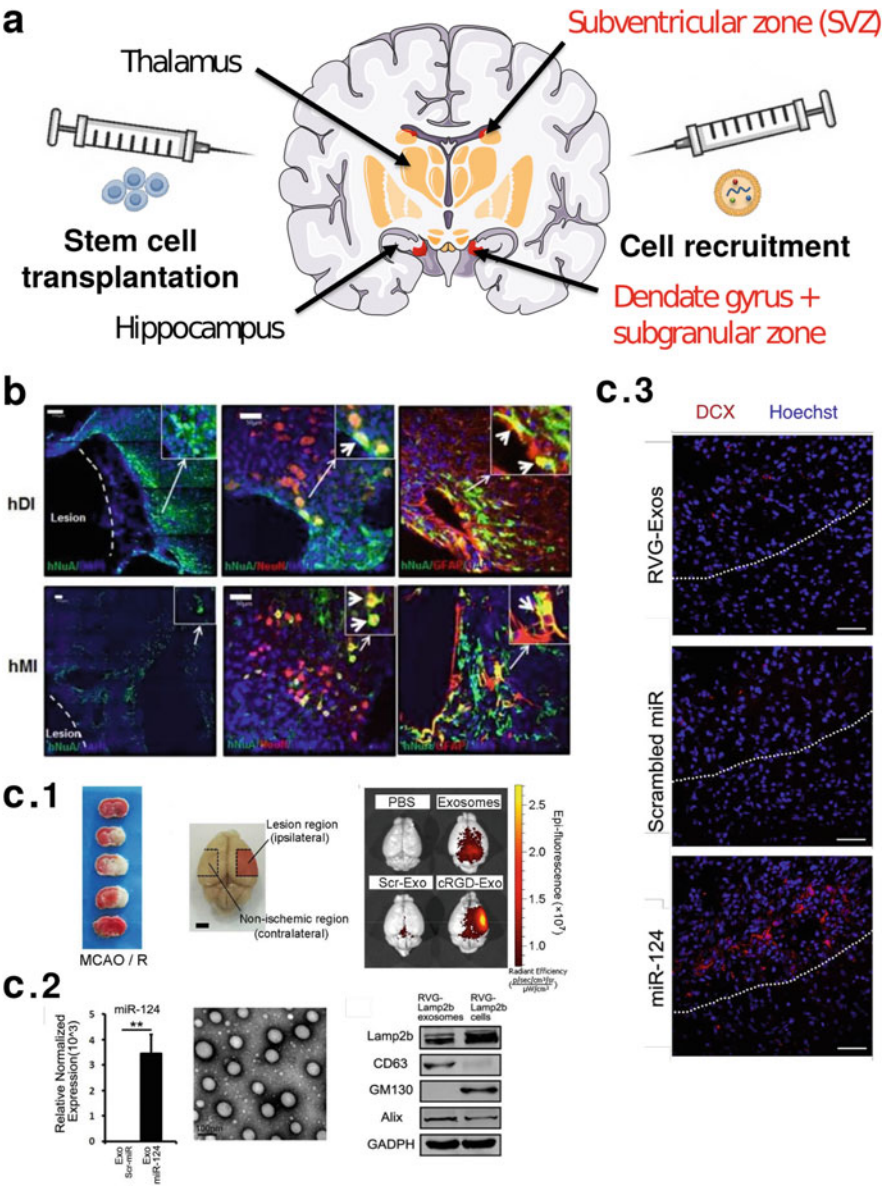

Fig. 8.3 Remarkable strategies for brain tissue regeneration. (**a**) Schematic representation of a coronal section of the brain, highlighting the putative reservoirs of NSCs capable of generating new neurons (red). These include the subventricular zone (SVZ), along the lateral wall of the lateral ventricles, and the subgranular zone of the dentate gyrus in the hippocampus. Because the adult brain is not capable of completely restore function after tissue damage, therapeutic approaches to promote neurogenesis consist of stem cell transplantation and the delivery of biomolecules to activate endogenous NSCs. Adapted from Servier Medical Art by Servier (http://smart.servier.com), which is licensed under a Creative Commons Attribution 3.0 Unported License. (**b**) Human dental stem cells (hDI) revealed superior performance than bone marrow-derived stem cells (hMI)

autologous transplantation. Dental pulp tissue offers very interesting prospects for neurogenesis because it is derived from the ectoderm/neural crest and endogenously mark for several neuronal markers [44]. In addition, dental pulp stem cells were demonstrated to differentiate into functionally active neurons and secrete neurotrophic factors, thus revealing superior therapeutic potential for brain regeneration after stroke than other stem cell sources (Fig. 8.3b) [37]. Clinical investigation of the beneficial effects of intravenously administered dental pulp stem cells is now underway in a Phase I clinical trial [45].

Other cell types of interest to improve neuronal cell function include immune cells, hematopoietic stem cells, and endothelial progenitor cells (EPCs). EPCs have the potential to reduce inflammation and apoptosis, to promote angiogenesis, and even to promote endogenous repair mechanisms. EPCs can be derived from the bone marrow and are classically defined by their surface expression of antigen CD34 [46]. Their presence at the ischemic core is associated with improved clinical outcome after stroke [47], due to their capability of remodeling brain vasculature and promoting angiogenesis [48], which peaks at the subacute phase [49, 50]. These promising results have supported the transplantation of $CD34^+$ cells for the treatment of ischemic stroke. Their clinical efficacy is currently under investigation in an ongoing interventional Phase IIa trial [51].

8.3.1.2 Cell Modulation Strategies

Although cell transplantation is a promising strategy for the generation of new neural cells and the replacement of lost neuronal circuitries with appropriate synaptic integration in the host tissue [52–54], there is still no definitive evidence with respect to clinical outcome improvements [40, 55]. This could be due to inefficient cell transplantation, which is still limited by their homing to the injured area [56] and cell survival on the damaged tissue microenvironment [57]. Numerous solutions have been tested to improve engraftment efficiency, from preconditioning or genetically modifying transplanted cells to adopting biomaterials (e.g. scaffolds) in order to facilitate their integration in the brain tissue. Recent approaches have

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Fig. 8.3 (continued) in promoting neurogenesis in rat brains 28 days after middle cerebral artery occlusion. This was demonstrated by immunohistochemical analysis of proliferating neurons ($NeuN^+$) and astrocytes ($GFAP^+$), which stained positive for human nucleus (hNuA). Scale bars = 100 μm. Adapted with permission from SAGE Publishing [37]. (c.1) EVs secreted by bone marrow-derived MSCs can be functionalized with brain-targeting peptides for local delivery of bioactive molecules. Reprinted from [38], Copyright (2018), with permission from Elsevier. (c.2) EVs are nano-sized vehicles which are characterized by the enriched expression of surface markers (e.g. CD63, Alix) and can be loaded with bioactive molecules by electroporation. (c.3) Delivery of microRNA-124 by EVs functionalized with targeting peptide RVG enhanced neurogenesis after ischemic stroke as demonstrated by the expression of the neuronal marker doublecortin (DCX) at the infarct site 7 days after administration. Reprinted from [39], Copyright (2017), with permission from Elsevier

coupled the manipulation of stem cells with electrical stimulation, which led to enhanced neurogenesis and angiogenesis [58]. Furthermore, NSCs from the own patient can be modulated to enhance neurogenesis [59]. We have demonstrated that polymeric nanoparticles (NPs) could mediate delivery of bioactive molecules to the SVZ in order to control differentiation of NSCs and EPCs, as well as to promote cell survival and normalize inflammatory responses occurring during ischemia [60–62]. NP-based formulations are attractive systems for cell modulation due to their efficacy, biocompatibility, and chemical versatility. They can be rendered compatible with imaging techniques, such as MRI [63, 64], or responsive to external stimuli (e.g. light) to confer spatiotemporal control over drug release to the brain [65, 66].

Besides cell replacement in the damaged brain, stem cell-mediated regenerative processes after stroke have been attributed to a paracrine effect characterized by the release of trophic factors and genetic modulators that activate brain remodeling pathways [67]. These biomolecules were found to be enriched in extracellular vesicles (EVs), which are nano-sized mediators playing key roles in intercellular communication [68]. EVs provide a cell-free option to modulate neural repair and overcome some of the limitations inherent to stem cell transplantation, including their scarcity and immunogenicity, which not only affects cell survival and motility after transplantation but can also cause significant adverse effects. Therapeutic EVs can be produced by MSCs and their content can be modulated for the delivery of proteins, lipids, and nucleic acids to enhance endogenous repair mechanisms (Fig. 8.3c). For instance, we and others have identified a panel of microRNAs associated with good prognosis after ischemic stroke, which affected migration of $CD34^+$ cells and their angiogenic activity [48, 49]. Further investigation is warranted to understand the effects of cell source and culture conditions on EVs content and, therefore, in their therapeutic potential.

8.3.2 Brain Electrical Stimulation

In addition to replacing damaged tissue with new cells, neurological functions can be restored after stroke by restructuring and rewiring functional networks [69, 70]. These restructuring processes are mainly due to the sprouting of spared axons, which innervate the affected regions, and create new neuronal circuitry [71, 72]. However, the brain alone does not have enough capacity to regenerate and reprogram neuronal circuits to the same complexity as that prior to stroke. Several strategies have been employed to maximize the chances of restoring sensory and motor functions by reestablishing neuronal connections [73, 74]. For instance, electrical stimulation of specific regions of the cortex has been explored to reorganize neural circuitry and restore brain functions after stroke (Fig. 8.4) [75]. Compared to pharmacological therapies, which can indiscriminately affect all neurons in the brain, this strategy allows for fewer adverse effects and much lower treatment associated costs [76]. Nevertheless, this has been employed mainly in

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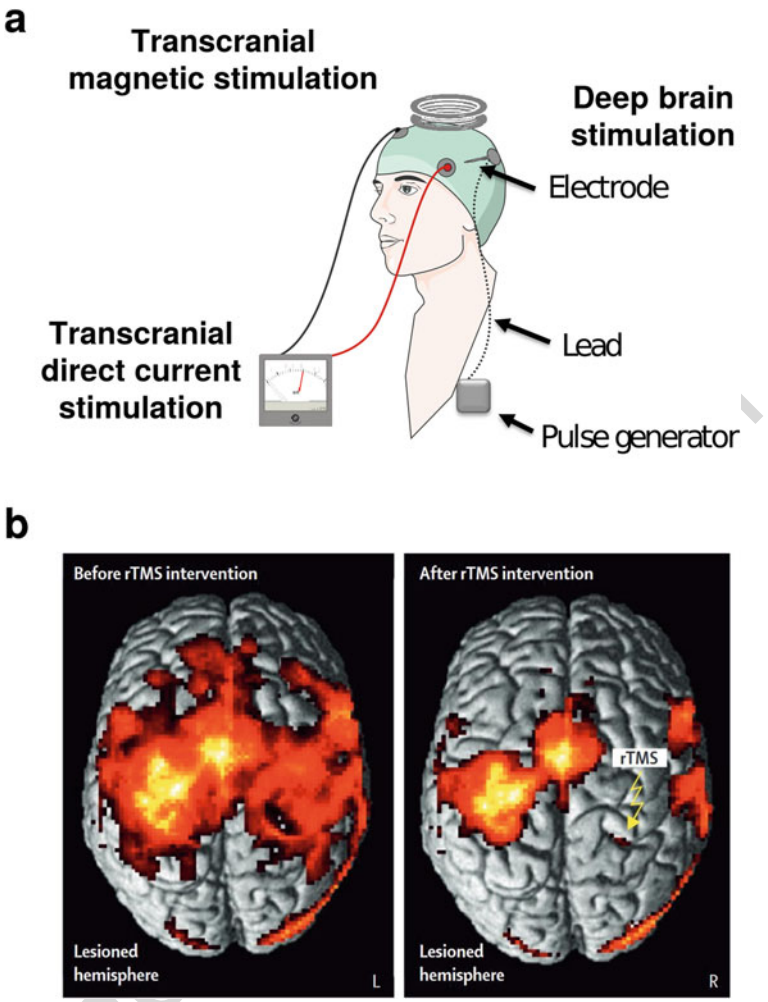

Fig. 8.4 Neuromodulation strategies for the management of stroke. (**a**) Schematic representation of stimulation modalities to modulate brain activity. Non-invasive modalities such as tDCS and transcranial magnetic stimulation (TMS) have been more frequently employed in the clinic. Adapted from Servier Medical Art by Servier (http://smart.servier.com), which is licensed under a Creative Commons Attribution 3.0 Unported License. (**b**) Functional MRI revealed that repetitive TMS of the contralesional primary motor cortex at 1 Hz inhibited excessive neural activity, which was associated with significant functional improvements. Reprinted from The Lancet [77], Copyright (2014), with permission from Elsevier

patients with significant neurological impairment. Considering the extensive tissue damage in these patients, neuromodulation has been primarily performed using minimally invasive techniques.

8.3.2.1 Transcranial Stimulation

Non-invasive modalities such as transcranial direct current stimulation (tDCS) and functional electrical stimulation (FES) consist in the application of electrodes on the skin surfacing the target region of interest. Typically in tDCS, one electrode targets the primary motor cortex, whereas the other acts over the contralateral supraorbital region. Based on the choice of anodal or cathodal electrodes [78], tDCS can induce either long-term potentiation or depression of neuronal activity, respectively, by modulating sodium- and calcium-dependent channels, as well as the NMDA receptor activity [79–81]. Although tDCS was shown to have an effect on upper limb functions in stroke patients, this occurred mostly during follow-up treatments, raising doubts about its long-term clinical efficacy [82].

On its turn, FES elicited moderate improvement in limb function by promoting muscle movement and mobility [83]. Due to the dissipation of the delivered current through the skull, high voltages are required to penetrate the brain tissue with enough power to activate neurons [84]. However, as high voltages were reported to cause patient discomfort, they were replaced by magnetic fields which have greater penetration depth [84]. Fast-oscillating magnetic fields along a copper coil external to the skull generate a strong electric current that can be directed to the motor cortex [84]. Specifically, transcranial magnetic stimulation (TMS) has been applied in the chronic setting of stroke in a strategy for interhemispheric inhibition [85–87]. It consists of exciting the ipsilesional primary motor cortex with high frequencies (>5 Hz) [88–90], whereas the contralesional primary motor cortex is inhibited using low frequencies (<1 Hz) [79, 91]. Other parameters such as stimulation time, coil shape, and magnetic field strength have been optimized to regulate cortical activity [79, 92, 93]. Despite some promising results particularly in the management of discrete neuropsychiatric conditions [94], magnetic stimulation of the brain and peripheral nerves is still at an early stage, and thus it has little clinical evidence of functional improvement in stroke patients [95, 96]. It is still unclear which protocol is more effective for improving motor function after stroke, given the lack of randomized controlled trials and small sample sizes [96]. New protocols have emerged, including the application of intermittent or continuous bursts of even higher frequencies than conventional TMS, thus requiring lower intensities [97, 98]. Such a variety of stimulation protocols warrants careful design of clinical trials to validate their safety and efficacy after stroke.

8.3.2.2 Deep Brain Stimulation

Although more invasive, modalities such as deep brain stimulation (DBS) are clinically well-established in movement disorders such as Parkinson's disease, and enable the stimulation of target regions with significant reproducibility [76]. DBS addresses the aforementioned issues of transcranial stimulation by implanting electrodes in regions adjacent to the target site [99]. Medical devices performing electrical stimulation have been tested in the clinic since the 1950s [100] and

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are successfully employed in the management of several neurological disorders where pharmacological options alone are inefficient, such as epilepsy, dementia, Alzheimer's disease and Parkinson's disease [99–101]. Currently, DBS is approved for the treatment of refractory Parkinson's disease, essential tremor, dystonia, obsessive–compulsive disorders, and drug-resistant partial onset epilepsy [102]. In the context of stroke, two objectives may arise from the use of DBS: the symptomatic treatment of extrapyramidal signs, following the same paradigm as in parkinsonian disorders, and the more conceptual goal of recovering brain function. First clinical evidence compiling several trials with small cohorts suggests that DBS could enhance motor status in stroke patients, particularly from disorders such as tremors, dyskinesia, and dystonia [103]. Such signs and symptoms represent post-stroke maladaptive responses where DBS could potentially have a role. In all these conditions, external electrical fields are thought to activate voltage-sensitive ion channels in neurons, which in turn generate chemical or electrical depolarization at their membranes, with subsequent release of neurotransmitters. As a result, irregular firing patterns in brain regions can be precisely modulated according to stimulus parameters such as signal amplitude, frequency, and duration [101].

Nevertheless, electrical stimulation performed by clinically approved DBS devices is experienced by all local cells, not only the targeted neurons. Other cell types including glia, fibroblasts, endothelium, and immune cells can also respond to these electrical cues, with significant effects in their phenotypes [104]. This could have an impact on the overall process of restoring brain function after stroke. Interestingly, transmembrane voltage for each cell type was associated with their differentiation state, with stem and proliferative cells being less polarized than terminally differentiated cells [104]. Hence, electrical stimulation could force membrane depolarization in neurons and glial cells that populate the infarcted area after stroke and promote tissue regeneration. *Post mortem* analysis showed that chronic stimulation (0.5–6 years) of the subthalamic nucleus enhanced neurogenesis in the neighboring SVZ in patients suffering from Parkinson's disease [105]. These findings encourage the investigation of potential in situ brain tissue regeneration following electrical stimulation. Yet no clinical trials to date have specifically demonstrated such effect, which could be attributed to the advanced disease progression by the time patients enroll in these studies [106].

8.3.2.3 Limitations of Deep Brain Electrical Stimulation

Indiscriminate stimulation of brain regions through conventional electrical stimulation devices might result in significant adverse effects, as reported in approximately 50–60% of patients and, in most cases, more than once [107–109]. Some of the most common causes of failure were improper electrode localization, inefficient device programming, infections, and hemorrhages resulting from surgical implantation [110]. Electrode positioning can be corrected with the guidance of imaging techniques (e.g. MRI, CT), while correct device programming overcomes issues such as overstimulation of undesired cells with high frequencies, which may

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impair physiological neuronal communication [111]. The recent development of closed-loop devices that adjust their stimulation parameters according to electrophysiological information recorded in real time paves the way for multifunctional neural interfaces, with further improvements expected in the following years [112]. The remaining caveats related to the implantation of DBS devices include their poor long-term stability and need for multiple surgeries to replace the electrodes.

Conventional electrodes are typically made of metals such as gold and iridium [113]. Metallic conductors are utilized because of their capability to readily mediate charge transfer between electrons at their interface with ions from the surrounding tissue (Fig. 8.5a). Most metals conduct electricity based on local reduction and oxidation reactions at the electrode surface, in a process known as Faradaic charge conduction. Repeated redox reactions at the metallic surface generate a hydrated oxide film that dramatically increases the amount of electric current that can be transferred to the adjacent tissue [113]. Although this electrochemical process is mostly reversible, changes in the electrolyte composition at the interface with the tissue can limit the rate of Faradaic reactions that can be performed without irreversibly modifying the material. Otherwise, not only the electrode can be degraded but also induce oxidative stress to the surrounding tissue. Conversely, capacitive charge conduction is a more desirable feature for implanted electrodes, since it involves solely the redistribution of charges at the electrode–electrolyte interface, thus avoiding redox reactions. However, capacitive materials such as titanium nitride suffer from limited charge injection capacity [113]. Pseudocapacitive materials such as platinum and its alloys with iridium have become then clinically adopted because they combine both Faradaic and capacitive conduction, hence increasing charge injection while minimizing redox effects [113]. For further details on electroactive materials with large charge capacity, readers are referred to Chap. 5 in this book.

Alongside charge transfer processes, the mechanical properties of the implanted materials are of upmost importance. Despite considerable efforts in the design of sterile, non-toxic materials with long-term chemical and electrical stability, they tend to trigger foreign body response because of their rigidity (>1 GPa) compared to the soft brain tissue (<10 kPa) (Fig. 8.5b) [118]. Mechanical mismatch of the implant promotes adverse biomechanical interactions leading to the formation of glial scars at the electrode interface as soon as few weeks after surgical implantation [118]. Ultimately, the efficacy of electrical stimulation and recording is dampened by the increased distance between the electrode and the target cells, as well as the impedance derived from the scar tissue [119]. Although device architecture can be engineered to minimize biological impact by decreasing local strain imposed by the electrodes [120], there is a clinical need for biocompatible electrodes that can be seamlessly integrated in the brain microenvironment. Electrodes can be incorporated in soft polymer mesh electronics (Fig. 8.5c), which facilitate their implantation by direct injection into the target brain region [115]. Besides being minimally invasive, mesh electronics are mechanically compliant to the brain tissue and, thus, more biocompatible, showing in vivo stability of up to 1 year without gliosis.

Additional challenges for neural interfaces include targeted stimulation of specific sites without affecting other physiological functions. These devices should

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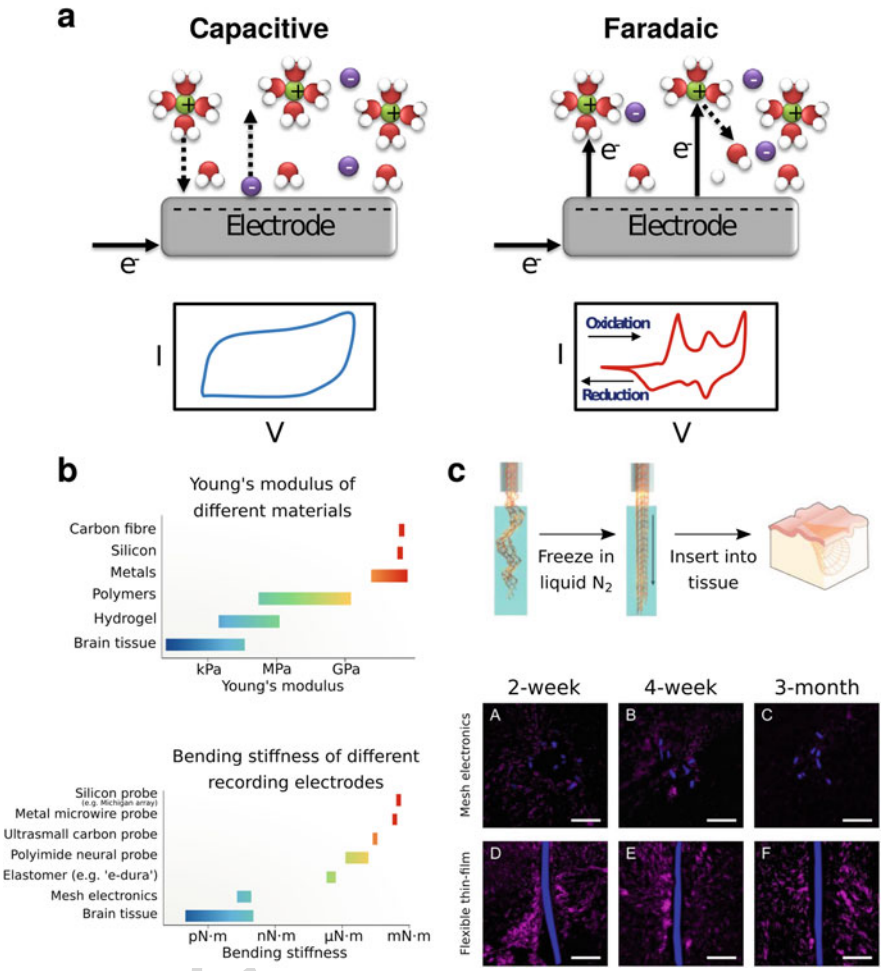

Fig. 8.5 Material properties determine long-term device biocompatibility and performance. (**a**) Electrical stimulation performed by electrodes depends on their electronic properties. Upon injection of electric current, capacitive materials such as titanium nitride, carbon nanotubes, and graphene generate a double layer at the electrode–electrolyte interface, attracting adsorbed water molecules and ionic species to the electrode surface [113, 114]. Because this process solely involves charge redistribution, the amount of charge injected from the electrode is limited by its surface. Although they enable greater amount of charge injected to the electrolyte, iridium oxide and PEDOT mediate Faradaic processes, which consist of the ejection of electrons from the electrode, leading to changes in the electrolyte composition and pH adjacent to the electrode [113]. Platinum and its alloys are attractive for brain stimulation because they combine capacitive and Faradaic processes, which result in higher charge injection with limited electrode degradation. Although these pseudocapacitive materials generate double layer charging, Faradaic processes may occur when specifically adsorbed ions react with the electrode surface [113]. (**b**) Typically used materials for implanted electrodes such as silicon, carbon, and metals are very rigid compared to brain tissues, presenting extremely high Young's moduli and bending stiffness values. Adapted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Reviews Neuroscience [115]. Copyright© 2019. (**c**) Mechanically compliant mesh electronics

be also capable of recording their physiological environment in order to coordinate neural stimulation parameters [119].

8.3.3 *Optogenetic Neuromodulation*

Exploring the intrinsic electrical properties of neurons, electrical stimulation has remained one of the main strategies to restore functional activity. However, because of its invasiveness, alternative approaches to DBS are being developed. One of the most promising is optogenetics, which combines light and genetic techniques to control and/or monitor cellular activity [121]. Although light has long been known to alter the behavior of neurons [122], this effect was only exploited in 2005, following their genetic modification with light-sensitive opsins [123]. Channelrhodopsins (ChR) are rapidly gated light-sensitive cation channels, commonly expressed in algae [124], and have provided unprecedented control over neuronal activity in well-defined neuronal populations with temporal precision. Upon light exposure, neuronal depolarization can be employed to investigate the functions of specific neurological circuitries and the mechanisms underlying neurological disorders [125, 126]. Even though optogenetics has been used mainly as a tool for neuroscience research in animals, therapeutic applications of this technology are under investigation [127–129].

Optogenetic tools have been applied in preclinical models of stroke (Fig. 8.6). In combination with voltage-sensitive dyes, the plasticity of the somatosensory cortex could be monitored after stroke, helping not only to understand the functional impact of the infarction but also to map potential regions of interest for stimulation [132]. Recovery of sensorimotor functions could be achieved after optogenetic stimulation of unaffected regions surrounding the infarcted cortex, such as corticospinal and thalamocortical neurons [130, 133]. In particular, stimulation of the ipsilesional primary motor cortex could contribute to functional recovery after stroke [129]. Repeated stimulation significantly improved neurovascular coupling and enhanced neuronal plasticity in the contralesional cortex. The cerebellum was also demonstrated to be a powerful target for brain stimulation due to the widespread activation of multiple motor and sensory regions via neuronal projections to the thalamus [134, 135]. All these studies have reported that optogenetic stimulation promoted axon growth and subsequent neuronal projections to the damaged site to

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Fig. 8.5 (continued) are attractive for brain implantation owing to their long-term biocompatibility and minimal inflammatory response. Immunohistochemical staining for Iba-1 (magenta) demonstrated that mesh electronics can be implanted in mice brains for several months and seamlessly integrate in the brain tissue with minimal glial response. Implanted probes were pseudo-colored blue. Scale bars = 100 μm. (c-top) Adapted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Reviews Materials [116]. Copyright© 2017. (c-bottom) Reprinted from [117], with permission from the National Academy of Sciences

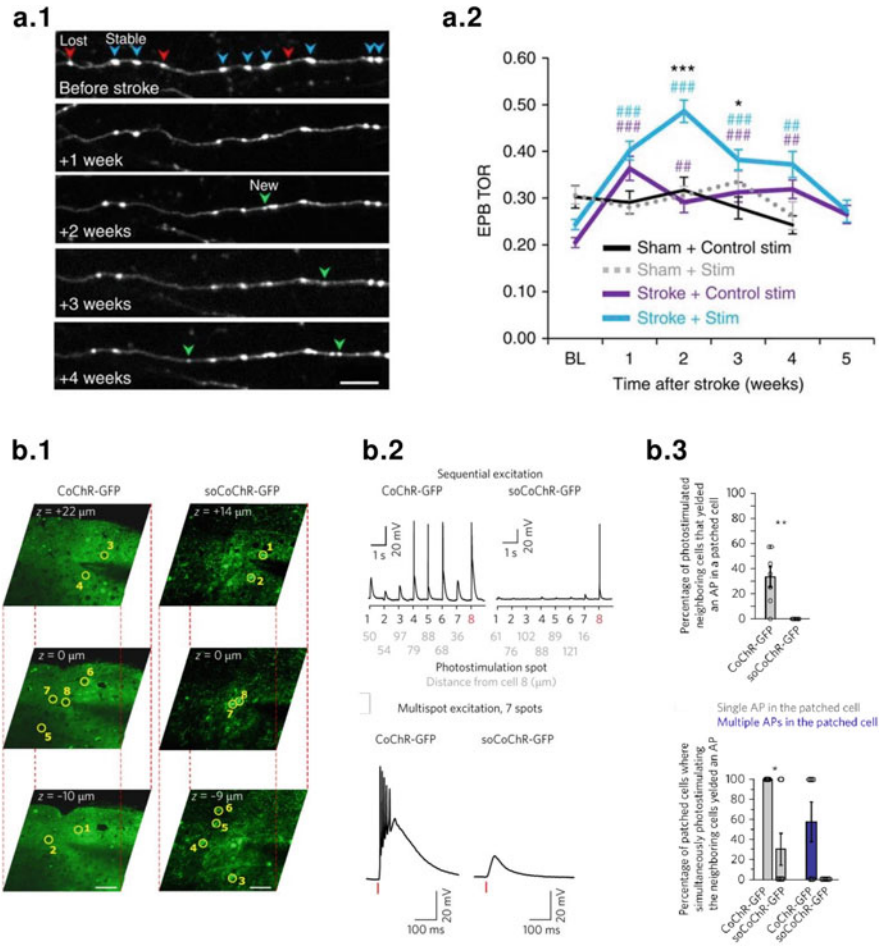

Fig. 8.6 Optogenetic stimulation for the treatment of stroke. (**a**) Optogenetic stimulation of ChR2-expressing thalamocortical neurons for up to 4 weeks after ischemic stroke significantly contributed to the formation of synaptic boutons, which play an important role in learning and memory processes. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Communications [130]. Copyright© 2017. (b.1) Soma-targeted opsins (soCoChR) are selectively expressed in the cell body of neurons. (b.2) Precise activation of soCoChR neurons by two-photon microscopy (λ = 1030 nm, 100 $\mu W/\mu m^2$) without affecting neighboring cells. (b.3) Engineered opsins enabled unprecedented precision over the stimulation of single cells, yielding well-defined action potentials in a given patched cell with minimal detection of action potentials from neighboring cells. Adapted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Neuroscience [131]. Copyright© 2017

remodel neural circuitry. A recent avenue of research resides in the possibility of enhancing neurogenesis in the SVZ. Considering that the striatum has neuronal projections to the physically adjacent SVZ, optogenetic stimulation of striatum glutamatergic neurons enhanced regeneration and functional recovery after ischemic stroke by evoking membrane currents and calcium influx in proliferating SVZ neuroblasts [136].

These promising results are encouraged by technological advances to enhance control over neuronal stimulation. While channelrhodopsins enable precisely timed depolarization of neurons, halorhodopsins derived from archaeal species can be stimulated with light of the same wavelength to hyperpolarize neurons [137]. The combination of these two rhodopsins can be used to accurately and bidirectionally control neuronal activity and cells native spiking patterns. Furthermore, spatiotemporal resolution could be enhanced by engineering opsins to potently respond to short light pulses (<1 ms), enabling single-cell stimulation by two-photon microscopy [131]. Other strategies to achieve spatiotemporal resolution over optogenetics include conditional expression of opsins using cell-specific promoters [138], which can be specifically activated using gene editing tools such as the Cre-*loxP* technology [139–141]. Because some cell-specific promoters have a weak transcriptional activity resulting on reduced levels of opsins in the cell membrane, Cre recombinase can be expressed in a cell-specific manner to enable expression of rhodopsins under the control of stronger ubiquitous promoters. Thus, optogenetic stimulation is controlled spatiotemporally by modulating the activity of Cre recombinase in specific cells, through either chemical [142, 143] or light-inducible [144, 145] Cre-*loxP* recombination systems.

Nevertheless, optogenetics faces considerable hurdles toward its clinical translation. One of them is the requirement of using either blue or green light as a trigger. Since visible light poorly penetrates biological tissues, invasive light sources such as fiber optics and light-emitting diodes have been applied in preclinical models, which may damage local tissues due to the heat dissipated from the light emission point [146]. Recently, a step-function opsin was engineered to respond to blue light with enhanced sensitivity and slower kinetics, which enabled transcranial activation owing to neuron depolarization for longer periods of time. Prolonged light accumulation compensates for its dissipation across biological tissues, allowing for transcranial stimulation in deeper regions of the brain down to 5 mm [147].

Considering the minimal absorbance of hemoglobin and water in this region (650–900 nm), the use of near-infrared (NIR) light is an attractive alternative due to its minimal scattering in biological tissues. NIR light not only penetrates deeper than visible light (up to 2 cm), but can also be less attenuated by the human skull (approximately, 0.5–5% of emitted light) [148]. For instance, lanthanide-doped up-conversion nanoparticles (UCNPs) have enabled deep tissue activation of rhodopsins by emitting visible light after exposure to NIR radiation [149–152]. These nanoparticles have promising optical properties including low autofluorescence background and minimal photobleaching and heat-mediated photodamage. Hence, UCNPs enable safer and minimally invasive stimulation compared to the use of NIR radiation alone [153] or combined with plasmonic nanoparticles such

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as gold nanorods to activate heat-sensitive proteins [154]. Moreover, UCNPs can act as remote actuators for transcranial NIR-activation of neuronal depolarization [149, 151, 155], enabling control over animal behavior in optogenetics studies. Finally, their chemical composition can be tuned to modulate light emission in order to selectively activate different channelrhodopsins and enhance the control over specific neural circuits [156]. These strategies open new opportunities to simultaneously control cell activity with spatiotemporal resolution and monitor neural circuits over time to improve recovery. However, the need for long-term expression of light-sensitive proteins, which is typically achieved by lentiviral vectors [128], carries numerous ethical and safety concerns regarding the possible genomic integration of undesired gene products after transfection, as well as potential adverse immune responses.

8.3.4 Coupling Optical and Electrical Stimulation of the Brain

Safety concerns related to the clinical use of optogenetics have prompted the investigation of numerous strategies to circumvent the need for genetic modification, while maintaining the capacity of specifically stimulating neurons with unprecedented resolution. This could be achieved by using photoactive nanomaterials and surfaces that generate an electric field when exposed to light, thus resulting in localized neuronal stimulation. This would avoid the need of implantable energy sources commonly used in DBS and prolong device lifetime. Moreover, device implantation would be desirably less invasive, with minimal foreign body response compromising long-term performance. However, this approach has not been investigated in preclinical stroke models yet because there are important biocompatibility considerations to minimize potential adverse effects in patients suffering from severe brain trauma. The section below explores the use of innovative polymers and nanomaterials, and the potential integration of light-responsive materials in such devices.

8.3.4.1 Novel Polymeric Materials for DBS

A main avenue of research consists of the design of minimally invasive devices using biodegradable materials (Fig. 8.7). These devices are based on biocompatible polymers, such as silk fibroin [159] and poly(lactic-co-glycolic acid) (PLGA) [160], and have been already developed for wireless electronic stimulation of peripheral nerves. This technology operates in a similar fashion to cochlear implants, where an external source of radiofrequency signals generates magnetic coupling with an antenna at the implanted device, which transduces that signal to electric current at the interfacing electrode. Although its application may be limited by the necessary power input to cross deeper regions such as those stimulated by DBS devices, the concept of bioresorbable devices is attractive for rehabilitation regimes in stroke because it avoids an additional surgical procedure to remove them. For instance,

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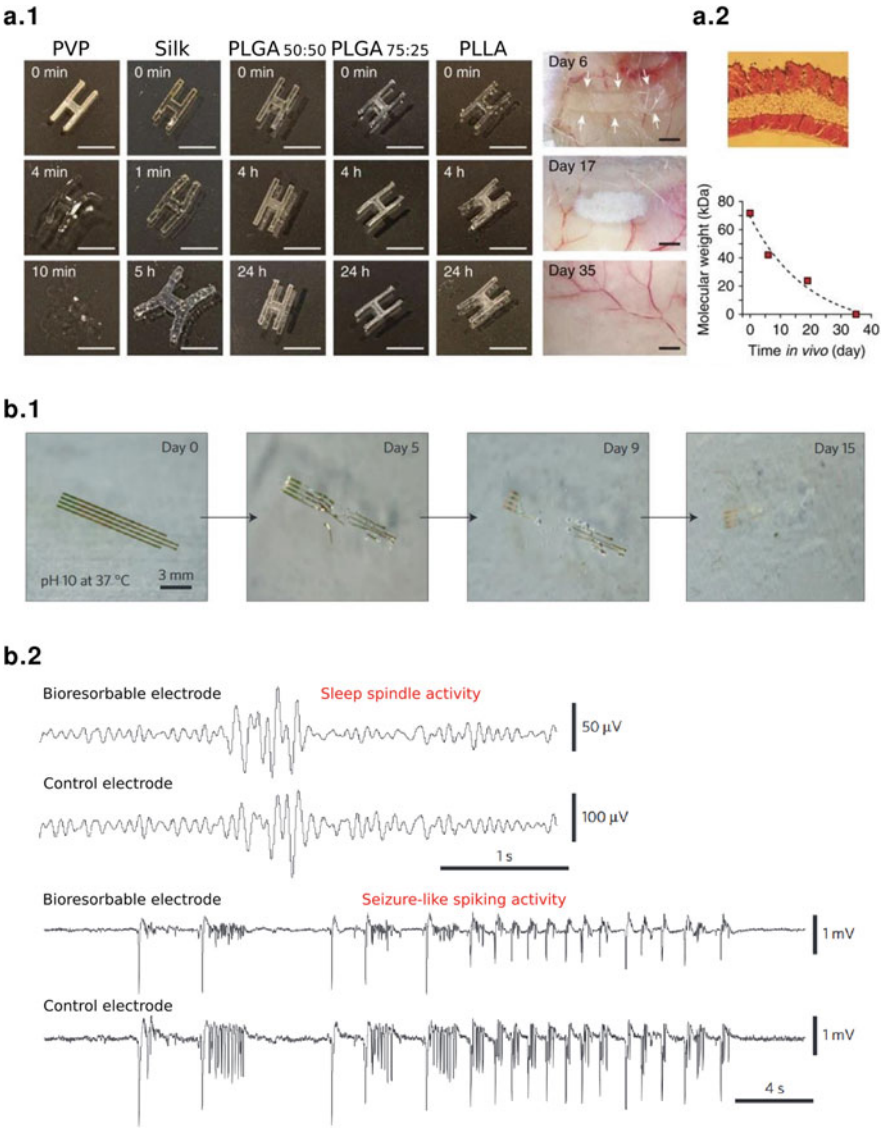

Fig. 8.7 Biodegradable electrodes enable transient monitoring and stimulation of the brain. (**a.1**) Biodegradability of silicon-based electrodes was tuned by adjusting the composition of PLGA films (50:50), in order to maintain their structural properties in phosphate buffer saline for several days, but were completely degraded within 35 days after subcutaneous implantation in a mouse model. (**a.2**) No signs of inflammatory response to the implant were observed. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Communications [157]. Copyright© 2016. (**b.1**) Dissolution profile in aqueous buffer solution (pH 10) at 37°C and (**b.2**) electrophysiological recording of cortical activity in rat brains during sleep and drug-induced epilepsy, compared to commercial stainless steel microwire electrodes. Silicon-based electrodes exhibited high signal-to-noise ratio. Adapted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Materials [158]. Copyright© 2016

silicon-based electrodes deposited on PLGA films recorded electrophysiological information from the rat cortex with comparable performance to clinically used electrodes [158], as well as intracranial pressure and temperature [161]. Other biocompatible polymer substrates and device operation modalities are currently under investigation to ensure long-term safety and improved electrical stimulation over more conventional methods [162].

Alternatively to biodegradable materials, a variety of biopersistent materials are well-established in the medical device industry. Device miniaturization could minimize their biological impact in the CNS. However, this comes at the expense of greater impedance, which is highly undesired in neural interfaces due to increased noise in recording electrodes and decreased amount of current that can be injected in stimulating electrodes [113, 163]. Impedance can be also detrimental for electrode longevity and biocompatibility because of local generation of heat from stimulating electrodes and potential toxic by-products from electrochemical reactions [113]. Finally, platinum is sensitive to various imaging techniques, producing artifacts in CT and MRI and interfering with optogenetics tools due to its lack of transparency [163]. Transparent materials that are not comprised of heavy elements and have low magnetic susceptibility are therefore preferred.

Indium tin oxide (ITO) is a transparent and electrically conductive material that is well-known for its application in touchscreens and solar cells. Despite its attractive features, ITO is expensive and brittle, which limits the available area of the electrode for recording and stimulation [164]. Alternatively, ITO could be deposited on flexible substrates such as parylene, poly(dimethylsiloxane) (PDMS), polymethylmethacrylate (PMMA), polyimide, and SU-8 epoxy [120]. However, ITO deposition requires temperatures that are higher than the glass transition temperature of most flexible polymer substrates [165]. Moreover, ITO has reduced optical transmittance toward the ultraviolet (UV)/blue and IR regions, maybe unsuitable for optogenetics. Although less conductive than ITO, flexible polymers such as poly(3,4-ethylenedioxythiophene) (PEDOT) surpass these challenges (Fig. 8.8a) [166]. PEDOT is a pseudocapacitive polymer stabilized in aqueous formulations by poly(styrenesulfonate) (PSS), which is also important in charge transfer processes resulting in the oxidation of PEDOT [113]. Despite its high electrical conductivity and low impedance [166], PEDOT:PSS lacks long-term stability in physiological milieu and delaminates from its substrate at higher charge densities [113], thus precluding its application in high-frequency recording and stimulation (Fig. 8.8b).

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8.3.4.2 Novel Nanomaterials for DBS

Aiming device miniaturization, nanomaterials have been increasingly applied either as an electrode coating for already existing devices or as electrodes themselves (Fig. 8.8b–c) [163, 170]. Owing to the network comprised by π electrons resulting from the sp^2 hybridization of carbon atoms, carbon nanomaterials such as carbon nanotubes (CNTs) and graphene have emerged as promising candidates for neural interfaces due to their high capacitive charge conductivity and physicochemical

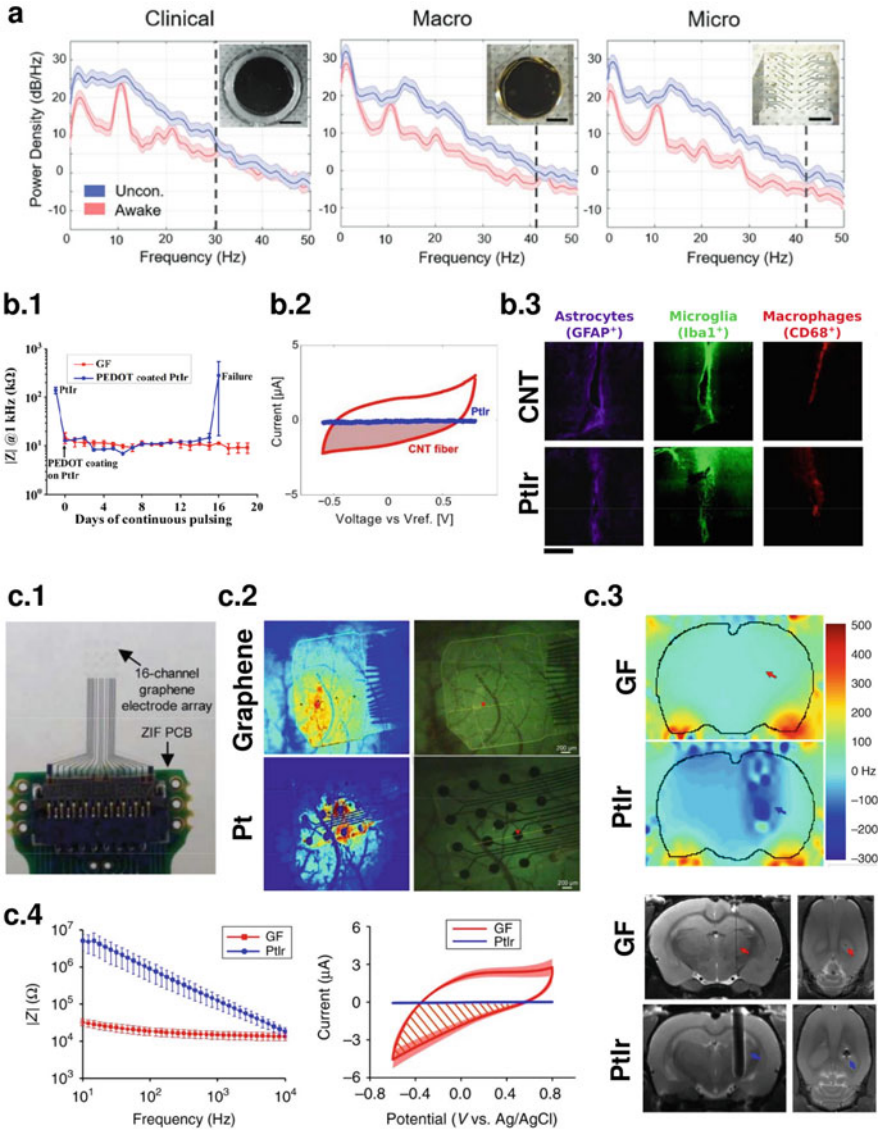

Fig. 8.8 Optically compatible materials for brain stimulation. (**a**) PEDOT:PSS electrodes showed comparable electrocorticography differences to clinically used platinum electrodes in recording brain activity of awake and unconscious rats. PEDOT:PSS maintained its sensitivity irrespective of electrode size, thus enabling device miniaturization. Reproduced from [166], with permission from John Wiley and Sons. © 2017 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (**b.1**) PEDOT:PSS-coated platinum–iridium (PtIr) electrodes show poor stability under prolonged continuous overpulsing at 1 kHz, demonstrated by the increased impedance comparable to uncoated PtIr electrodes. (**b.2**) CNT fibers mediated capacitive charge conduction and showed greater stability, (**b.3**) but their rigidity triggered significant glial response 6 weeks after implantation. (**c.1**) Transparent graphene-based electrodes enabled multimodal imaging to monitor brain activity

stability [163]. For instance, microelectrodes containing vertically aligned CNTs enabled highly sensitive electrochemical measurements and precise stimulation of brain regions at the nanotube tip [168, 171, 172], with CNT coatings enhancing the electrode stability [172, 173]. In addition, the well-defined electronic energy levels of single-walled CNTs (also known as Van Hove singularities) could guide the design of electrodes with minimal light-induced artifacts during optogenetics stimulation and record electrophysiological activity with high fidelity [174]. However, biomedical research involving CNTs has become somewhat controversial [175]. For instance, a type of long multi-walled CNT fibers with high aspect ratio (MWCNT-7) has been classified as "potentially carcinogenic to humans" based on extensive preclinical evidence of tumor formation due to excessive fibrotic and inflammatory responses [176].

Sharing similar electronic features with CNTs, graphene has emerged as a strong candidate for the development of neural interfaces [114]. Despite its potentially slow degradation profile [177], graphene is more flexible and biocompatible than CNTs, evidenced by the lack of significant fibrosis in multiple tissues after different administration routes [178, 179]. In fact, graphene substrates were shown to improve neural cell growth and differentiation by potentiating electric circuits [180–182]. Moreover, the application of graphene as surface coatings not only protected metal electrodes from corrosive electrochemical reactions at their surface, but also shielded them from electromagnetic interference during MRI, hence minimizing image artifacts [183]. Such compatibility with functional MRI has facilitated the mechanistic study of the therapeutic effects of DBS in Parkinsonian rats using graphene-based fiber electrodes (Fig. 8.8c) [167].

Altogether, these properties enabled graphene to be employed in flexible interfaces for multimodal imaging, which couple recording neural activity with high sensitivity and spatiotemporal resolution. For instance, graphene-based transistor arrays designed for electrocorticography were demonstrated to map electrical activity in the brain with greater spatial resolution and lower electronic noise than clinically used platinum and gold [184, 185]. Furthermore, a neural interface comprised of graphene-based sensing and stimulating electrodes was shown to regulate thalamocortical circuits and effectively correct abnormal epileptic activity using high-frequency discharges, after epidural implantation [186]. Graphene-based electrode arrays have been also developed to couple optogenetics stimulation with electrophysiological recording [165, 187]. Despite superior performance compared

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Fig. 8.8 (continued) and (**c.2**) minimal artifacts in fluorescence imaging compared to clinically used platinum-based electrodes. (**c.3**) Graphene fiber electrodes insulated with Parylene C enabled brain stimulation of the subthalamic nucleus of rat brains with minimal interference in MRI. (**c.4**) Graphene exhibits lower electrical impedance than PtIr and greater charge injection by capacitive charge conduction, thus demonstrating superior performance for brain stimulation. (b1,c3,c4) Adapted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Communications [167]. Copyright© 2020. (b2,b3) Adapted with permission from [168]. Copyright (2015) American Chemical Society. (c1,c2) Adapted with permission from [169]. Copyright (2018) American Chemical Society

to platinum, graphene electrodes could suffer from artifacts derived from photoelectric effects upon exposure to blue light. As these artifacts were mostly limited to the immediate vicinity of the irradiated electrode, this phenomenon was attributed to the photovoltaic effect, which is characterized by the generation of electric current upon light exposure. Also, similarly to what is commonly observed in metals, light-induced artifacts depended on incident laser power and exposure time. The observed light-induced artifacts could compromise the use of graphene electrodes in combination with optogenetics tools. Nevertheless, this intrinsic capability of generating electricity upon light exposure could offer a promising alternative to optogenetics by avoiding the need of genetic modifications. To this regard, Savchenko et al. discovered that graphene substrates could elicit cell contraction upon light stimulation [188]. Consistent with the aforementioned photoelectric effect, light stimulation elicited capacitive charge injection. In these studies, cellular activity was manipulated by adjusting light intensity rather than wavelength.

Alternatively, silicon nanowires (SiNWs) have been also recently explored toward the development of photoresponsive electrodes mediating optoelectronic stimulation of cardiomyocytes and neurons [189–191]. SiNWs convert light into electricity via photothermal and photoelectrochemical reactions catalyzed by atomic gold used to nucleate and generate these nanostructures. In addition, conductive polymers have been employed in the preclinical development of retinal implants and could provide a platform for optoelectronic stimulation [192]. Further investigation on their photosensitivity, as well as their long-term biocompatibility and stability, is warranted to determine their clinical applicability.

8.4 Conclusions and Future Perspectives

Recent improvements in critical care of acute ischemic stroke have saved the lives of millions of patients worldwide. However, most survivors experience noticeable deficits in neurological function, which could affect independence in their daily lives. Novel therapies and devices have been developed with the aim of resolving or attenuating these disabilities.

Stem cell transplantation has been the most investigated strategy to date for restoring brain functions. However, key factors determining the success of this strategy remain unknown. First, the influence of donor cell type and tissue origin for the transplant needs to be considered to ensure their integration in the injured brain site. Furthermore, the patient clinical history (e.g. age, sex, presence of comorbidities, and recent surgical procedures such as recanalization), delivery method for the treatment (e.g. intravenous, intra-arterial, and stereotaxic), and timeline may also play important roles in choosing the appropriate regime. Transplanted stem cells are more effective when delivered at early stages to modulate tissue regeneration and reintegration in the neuronal circuitry. However, the exacerbated immune response to traumatic injuries may limit their efficacy. In this sense, clinical evidence shows limited efficacy of stem cells in improving neuronal function after stroke. This could

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be explained by late interventions performed at subacute and chronic stages after stroke, when neuronal circuitry has been already reestablished [40, 70]. Further investigation is required to evaluate whether immune and angiogenic responses dominating the subacute stage could have a beneficial impact on neurogenesis and synaptogenesis [193, 194]. Considering the high cost of cell transplantation, the delivery of EVs arises as an attractive cell-free option to mimic some of the beneficial effects of stem cells. However, this therapeutic strategy requires further development and testing [68].

Medical devices for brain stimulation are expected to undergo significant technological development in the following years, following the clinical acceptance of different materials from the conventionally used metals as electrodes. Silicon- and graphene-based nanomaterials rank among the most promising candidates for bioelectronics, owing to their biocompatibility. However, current fabrication processes are laborious and involve high temperatures which are not conducive to their application in flexible polymer substrates. Cost-effective procedures such as inkjet printing should yield electrically conductive nanomaterials which can be formulated to facilitate their incorporation in soft interfaces, thus making them more accessible [195, 196]. Nonetheless, the effects of long-term exposure to these nanomaterials require extensive assessment of device biocompatibility along its life cycle, including the careful characterization of dissolution and/or degradation by-products. Covalent functionalization and chemical doping strategies will provide added control over nanomaterial biocompatibility and biodegradability for biomedical applications [197–200].

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