Chapter 9
Nanoneurology

Introduction

Neurology deals with the study and management of disorders of the nervous system. Considerable research is in progress in basic neurosciences and clinical neurology. The management is mostly medical. Many neurological disorders require surgical intervention, and the closely related specialty of surgical neurology or neurosurgery will also be considered in this chapter. There is a considerable scope for application of nanobiotechnology in neurology and hence the term nanoneurology (Jain 2009a). Nanobiotechnology has been applied for neurophysiological studies, diagnosis, neuropharmacology, and refinement of surgical tools (Jain 2012i). Neuroprotection is an important objective in treatment of diseases of the central nervous system (CNS).

Nanobiotechnology for Neurophysiological Studies

Use of Nanoelectrodes in Neurophysiology

Insulated microelectrodes are used in neurophysiological studies since 1950s with minor modifications. Single large neuron recordings are possible with electrodes in μm diameter range. For small neurons, it is worthwhile to have electrodes with nanoscale tips for recording. It is now possible to grind the bare tip of a tungsten microelectrode down to 100–1,000 nm and remove the insulation at the tip. A 700-nm tipped electrode was demonstrated to record well-isolated action potentials extracellularly from single visual neurons in vivo (Qiao et al. 2005). Experimental studies have shown that sub-100 nm silicon nanowires can be integrated into live cells without causing detrimental effects (Kim et al. 2007).

Transistor arrays of silicon nanowires with diameter of 30 nm and fabricated on transparent substrates can be reliably interfaced to acute brain slices (Qing et al. 2010). These can record across a wide range of length scales, whereas the transparent device chips only provide imaging of individual cell bodies. Combination of

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arrays with patch clamp studies enables identification of action potential signals. The result is a recording with high temporal and spatial resolution, as well as mapping of functional connectivity. This provides a powerful platform for studying neural circuits in the brain.

Nanowires for Monitoring Brain Activity

Electrical recording from spinal cord vascular capillary bed has been achieved demonstrating that the intravascular space may be utilized as a means to address brain activity without violating the brain parenchyma. Working with platinum nanowires and using blood vessels as conduits to guide the wires, researchers have successfully detected the activity of individual neurons lying adjacent to the blood vessels (Llinas et al. 2005). This can provide an understanding of the brain at the neuron-to-neuron interaction level with nonintrusive, biocompatible and biodegradable nanoprobes. This technique may one day enable monitoring of individual brain cells and perhaps provide new treatments for neurological diseases. Because the nanowires can deliver electrical impulses as well as receive them, the technique has potential as a treatment for Parkinson's disease (PD). It has already been shown that patients with PD can experience significant improvement from direct stimulation of the affected area of the brain. But the stimulation is currently carried out by inserting wires through the skull and into the brain, a process that can cause scarring of the brain tissue. By stimulating the brain with nanowires threaded through blood vessels, patients can receive benefits of the treatment without the damaging side effects. The challenge is to precisely guide the nanowire probes to a predetermined spot through the thousands of branches in the brain's vascular system. One solution is to replace the platinum nanowires with new conducting polymer nanowires. Not only do the polymers conduct electrical impulses, they change shape in response to electrical fields, which would allow the researchers to steer the nanowires through the brain's circulatory system. Polymer nanowires have the added benefit of being 20–30 times smaller than the platinum ones used in the reported laboratory experiments. They are biodegradable and therefore suitable for short-term brain implants.

Gold Nanoparticles for In Vivo Study of Neural Function

As a novel in vivo method to study interactions between gold nanoparticles AuNPs and the nervous system, negatively charged AuNPs, 50 nm in diameter, were injected into the CNS of a cockroach (Rocha et al. 2011). The charged nanoparticles affected the insect's locomotion and behavior but no significant effect on the life expectancy of the cockroach after 2 months of observation, apparently due to the encapsulation of AuNPs inside the insect's brain. This inexpensive method offers an opportunity to further understand how nanoparticles affect neural communication by monitoring insect activity and locomotion.

Nanodiagnosis and Nanoparticle-Based Brain Imaging

Nanodiagnostic technologies described in Chap. 4 are applicable to neurological disorders. Relation of various technologies to diagnosis of neurological disorders is shown in Fig. 9.1.

Applications of Nanotechnology in Molecular Imaging of the Brain

Some of the applications of nanobiotechnology in brain imaging are the following:

- Tracking of stem cells by tagging with nanoparticles such as SPIONs so they can be detected with MRI
- QDs for molecular imaging in cerebrovascular disorders
  - Early aneurysm detection and guide endovascular intervention
  - Imaging of vessels prone to spasm
  - To distinguish penumbra from infarction
  - To identify unstable arterial plaques for targeted intervention
- Early diagnosis of neurodegenerative disorders
- Early diagnosis of brain tumors

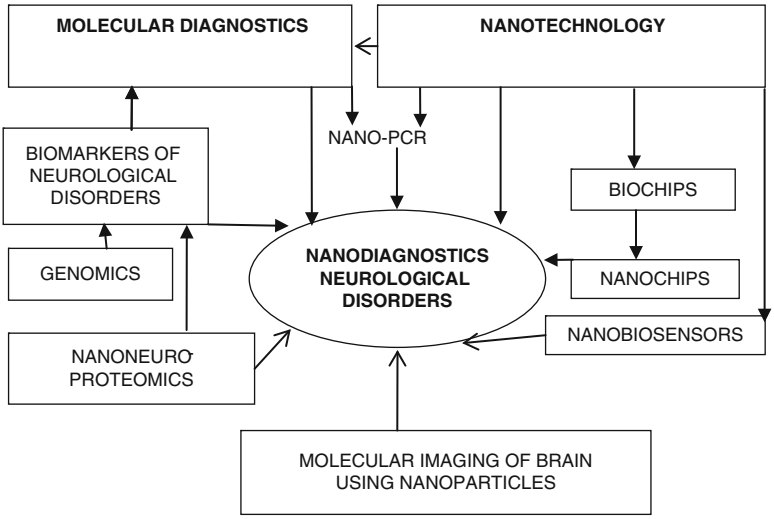

Fig. 9.1 Nanodiagnostics for neurological disorders (© Jain PharmaBiotech)

Nanoparticles and MRI for Macrophage Tracking in the CNS

Activated macrophages, acting in concert with other immune competent cells, are an index of inflammatory/immune reaction in CNS disorders such as multiple sclerosis, ischemic stroke lesions, and tumors. The MRI detection of brain macrophages defines precise spatial and temporal patterns of macrophage involvement that helps to characterize individual neurological disorders. Macrophage tracking by MRI with iron oxide nanoparticles has been developed during the last decade for numerous diseases of the CNS. Experimental studies on animal models were confirmed by clinical applications of MRI technology of brain macrophages. This approach is being explored as an in vivo biomarker for the clinical diagnosis of cerebral lesion activity, in experimental models for the prognosis of disease development, and to determine the efficacy of immunomodulatory treatments under clinical evaluation (Petry et al. 2007). Comparative brain imaging follow-up studies of blood–brain barrier leakage by MRI with gadolinium chelates, microglia activation by PET with radiotracer ligand PK11195, and MRI detection of macrophage infiltration provide more precise information about the pathophysiological cascade of inflammatory events in cerebral diseases. Such multimodal characterization of the inflammatory events should help in the monitoring of patients, in defining precise time intervals for therapeutic interventions, and in developing and evaluating new therapeutic strategies.

Nanoparticles for Tracking Stem Cells for Therapy of CNS Disorders

Cellular MRI using superparamagnetic iron oxide nanoparticles (SPION) can visualize and track cells in living organisms. MRI studies have been conducted in rat models of CNS injury and stroke to track stem cells that were either grafted intracerebrally, contralaterally to a cortical photochemical lesion, or injected intravenously (Sykova and Jendelova 2007). ESCs and MSCs were labeled with iron oxide nanoparticles (Endorem®) and human $CD34^+$ cells were labeled with magnetic MicroBeads (Miltenyi). During the first posttransplantation week, grafted MSCs or ESCs migrated to the lesion site in the cortex as well as in the spinal cord and were visible in the lesion on MRI as a hypointensive signal, persisting for more than 30 days. In rats with an SCI, an increase in functional recovery was noted after the implantation of MSCs or after an injection of granulocyte colony stimulating factor (G-CSF). Morphometric measurements in the center of the lesions showed an increase in white matter volume in cell-treated animals. Prussian blue staining confirmed a large number of iron-positive cells, and the lesions were considerably smaller than in control animals. To obtain better results with cell labeling, new polycation-bound SPIONs (PC-SPIONs) were developed. In comparison with Endorem, PC-SPIONs demonstrated a more efficient intracellular uptake into MSCs with no decrease in cell viability. These studies demonstrate that MRI of grafted adult as well as ESCs labeled with iron oxide nanoparticles is a useful method for evaluating cellular migration toward a lesion site.

Autologous bone marrow CD34+ cells labeled with magnetic nanoparticles have been delivered into the spinal cord via lumbar puncture in a study on patients with chronic SCI (Callera and de Melo 2007). One group received their own labeled CD34+ cells, whereas the others received an injection containing only magnetic nanoparticles without stem cells to serve as controls. CD34+ cells were labeled with magnetic nanoparticles coated with a monoclonal antibody specific for the CD34 cell membrane antigen. MRI showed that magnetically labeled CD34+ cells were visible at the lesion site as hypointense signals following transplantation, but these signals were not visible in any patient in the control group. This study shows that autologous bone marrow CD34+ cells labeled with magnetic nanoparticles, when delivered intrathecally, migrate into the site of injury in patients with chronic SCI and can be tracked by MRI. This shows the feasibility of treatment of SCI with intrathecal cell therapy.

Multifunctional NPs for Diagnosis and Treatment of Brain Disorders

Multifunctional NPs (MFNPs) are particularly suited for combining diagnostics with therapeutics of brain disorders. Tailoring the size, contents, and surface electronic properties through chemistry and physical methods within sub-200 nm nanoparticles will be key factors for using MFNPS (Suh et al. 2009). Functions such as directing neuronal growth and influencing stem cell differentiation for brain repair seem to be the next logical step in nanobiotechnology utilizing MFNPS. Studies involving stem cell differentiation and transplantation, neural implants, targeted drug delivery with real-time monitoring capabilities, and in vivo RNAi will be of great interest. Advances in neuroscience will arise from systematic investigations starting from synthesis to application where the efforts are focused on probing and understanding events occurring at the nano–bio interface.

Nanotechnology-Based Drug Delivery to the CNS

Delivery of drugs to CNS is a challenge, and the basics as well as various strategies are discussed in a special report on this topic (Jain 2012e). Molecular motors, operating at nanoscale, can deliver drugs to the CNS by peripheral muscle injection. An advantage is the use of nanomotors in native environment for intraneural drug delivery. The disadvantages are that this approach requires engineered molecular motors for use in cells and neurotoxicity may be a problem.

Nanoencapsulation for Delivery of Vitamin E for CNS Disorders

Vitamin E is used for the treatment of neurological disorders, particularly those where oxidative stress plays a role. Oxidative stress is an early hallmark of affected

neurons in Alzheimer's disease (AD). The antioxidant vitamin E provided limited neuroprotection in AD, which may have derived from its lipophilic nature and resultant inability to quench cytosolic reactive oxygen species (ROS), including those generated from antecedent membrane oxidative damage. Encapsulation into polyethylene glycol (PEG)-based nanospheres, which can enter the cytosol, improved the efficacy of vitamin E against Aβ-induced ROS (Shea et al. 2005). These findings suggest that nanosphere-mediated delivery methods may be a useful adjunct for antioxidant therapy in AD.

Nanoparticle Technology for Drug Delivery Across BBB

Currently most of the strategies are directed at overcoming the blood–brain barrier (BBB). Role of nanobiotechnology in overcoming BBB is described elsewhere (Jain 2012f). Very small nanoparticles may just pass through the BBB but this uncontrolled passage is not desirable. Most of the strategies described in this report for passage of drugs across the BBB can be enhanced by nanotechnology and some examples are the following (Barbu et al. 2009):

- Nanoparticles open the tight junctions between endothelial cells and enable the drug to penetrate the BBB either in free form or together with the nanocarrier.
- Nanoparticles are transcytosed through the endothelial cell layer and allow the direct transport of their therapeutic cargo.
- Nanoparticles are endocytosed by endothelial cells and release the drug inside the cell, as a precursor step to the transport of active ingredients, which occurs by exocytosis at the abluminal side of the endothelium.
- Nanoparticles, which combine an increased retention at the brain capillaries with adsorption onto the capillary walls, improve delivery to the brain by creating a concentration gradient that promotes transport across the endothelial cell layer.
- Drug transport is enhanced by the solubilization of the endothelial cell membrane lipids by surfactant, which leads to membrane fluidization (surfactant effect).
- Coating agents (such as polysorbates) inhibit the transmembrane efflux systems, i.e., P-glycoprotein.
- Nanoparticles induce local toxic effects at the brain vasculature, which leads to a limited permeabilization of the brain endothelial cells.

BBB represents an insurmountable obstacle for a large number of drugs, including antibiotics, antineoplastic agents, and a variety of central nervous system (CNS)-active drugs, especially neuropeptides. One of the possibilities to overcome this barrier is a drug delivery to the brain using nanoparticles. Drugs that have successfully been transported into the brain using this carrier include the hexapeptide dalargin, the dipeptide kytorphin, loperamide, tubocurarine, the NMDA receptor antagonist MRZ 2/576, and doxorubicin.

The mechanism of the nanoparticle-mediated transport of the drugs across the BBB at present is not fully elucidated. The most likely mechanism is endocytosis by the endothelial cells lining the brain blood capillaries. Nanoparticle-mediated drug transport to the brain depends on the overcoating of these materials with polysorbates, especially polysorbate 80, which seems to lead to the adsorption of apolipoprotein E from blood plasma onto the nanoparticle surface. The particles then seem to mimic low-density lipoprotein (LDL) particles and could interact with the LDL receptor leading to their uptake by the endothelial cells. After this, the drug may be released in these cells and diffuse into the brain interior or the particles may be transcytosed. Other processes such as tight junction modulation or P-glycoprotein (Pgp) inhibition also may occur. Moreover, these mechanisms may run in parallel or may be cooperative thus enabling a drug delivery to the brain.

The use of NPs to deliver drugs to the brain across the BBB may provide a significant advantage to current strategies. The primary advantage of NP carrier technology is that NPs mask the BBB limiting characteristics of the therapeutic drug molecule. Furthermore, this system may slow drug release in the brain, decreasing peripheral toxicity. Various factors that influence the transport include the type of polymer or surfactant, NP size, and the drug molecule. Use of metallic NPs such as AuNPs is associated with risk of neurotoxicity, and special precautions such as coating of NPs are required to prevent this. Other nanomaterials used for delivery across BBB include dendrimers, lipid NPs, liposomes, micelles, nanogels, PLGA, poly-ε-caprolactone, and polymeric NPs.

Polymeric nanoparticles have been shown to be promising carriers for CNS drug delivery due to their potential both in encapsulating drugs, hence protecting them from excretion and metabolism, and in delivering active agents across the BBB without inflicting any damage to the barrier (Tosi et al. 2008). Polymeric NPs for delivery across BBB should have the following ideal properties: biocompatible, nontoxic, nonthrombogenic, and nonimmunogenic (Martin-Banderas et al. 2011).

By designing well-controlled and appropriate preclinical and clinical translational studies, use of nanotechnologies for safely, efficiently, and specifically delivering drugs and other molecules across the BBB may prove one of their highest impact contributions to clinical neuroscience (Silva 2010). Two strategies for transporting drugs across the BBB are in commercial development: G-technology and LipoBridge.

G-Technology®

G-Technology® (to-BBB) platform utilizes nanoliposomes coated with glutathione-conjugated PEG to mediate safe targeting and enhanced delivery of drugs to the brain. Glutathione, an endogenous tripepeptide transporter, is highly expressed on the BBB. Intravenous injections of PEGylated liposomes are already on the market (Doxil), and high dosages of glutathione in supportive therapy in cancer as well. Glutathione, a natural antioxidant, is found at high levels in the brain, and its receptor is abundantly expressed at the BBB. Therefore, glutathione minimizes adverse effects such as adverse immunological reactions or interference with essential

physiological pathways. None of the other technologies for delivery of drugs to the brain have the favorable pharmacokinetic and safety profile of the G-Technology®. This technology utilizes an endogenous receptor-mediated endocytosis mechanism in combination with nanosized drug-loaded liposomes. This approach is unique in that it does not require drug modification and at the same time gives rise to metabolic protection during transport and increased bioavailability at the target site.

LipoBridge™ Technology

LipoBridge™ (Genzyme Pharmaceuticals) temporarily and reversibly opens tight junctions to facilitate transport of drugs across the BBB and into the CNS. LipoBridge itself forms a clear suspension of nanoparticles in water and can solubilize or stabilize some drugs, is nonimmunogenic, and is excreted unmetabolized. It has been demonstrated in several laboratories that intracarotid injections of a simple mixture of Lipobridge™ and model compounds or pharmaceutical actives can deliver these actives into one or both hemispheres of the brain allowing for increased concentration in a selected hemisphere. It can be administered orally as well as intravenously. LipoBridge has been used to administer anticancer drugs for brain cancer in animals. Safety clinical studies in humans are in progress.

Nanovesicles for Transport Across BBB

According to US patent application #20070160658, scientists at the Ben-Gurion University of the Negev, Israel, are developing a targeting moiety conjugated to the nanovesicle, which comprises a therapeutic composition. These nanovesicles are useful in treatment of a wide spectrum of disorders. This technology solves the problem of transport through the BBB by using nanovesicles that are able to cross the BBB and which carry the desired drugs by using a targeted delivery mechanism where the drug will be released from the vesicle in the brain. The drug to be delivered is encapsulated within stable nanosized vesicles (20–100 nm) possessing surface moieties that facilitate the release of the drug at target sites, such as the brain. The method of targeting is based on head groups that are selectively cleaved at the target site by enzymatic activity, thus releasing the encapsulated material primarily at the target organ. Injection of an encapsulated analgesic peptide, encephalin, into mice showed an analgesic effect comparable to morphine, while encephalin in its free form did not to penetrate the BBB and had no effect. Potential applications of this technology include cancer, pain, and neurodegenerative diseases such as Alzheimer's and Parkinson's. The advantages are as follows:

- Vesicles are stable and flexible, allowing penetration through biological barriers.
- Unique surface chemistry allows the incorporation of selective targeting proteins.
- V-Smart targeting mechanism allows better precision in drug delivery by unloading drug from the vesicle only in predetermined location characterized by unique enzyme which causes drug release from the vesicle.

Nanotechnology-Based Drug Delivery to Brain Tumors

The focus of this section is glioblastoma multiforme (GBM), a primary malignant tumor of the brain. Treatment of GBM is one of the most challenging problems. Surgery remains the basic treatment in which the bulk of the tumor is removed and the peripheral infiltrating part is the target of supplementary treatments. GBM is not easily targeted but advances in nanobiotechnology have improved the prospects of delivery of therapeutics to GBM (Jain 2007a).

Multifunctional Nanoparticles for Treating Brain Tumors

One approach combines two promising approaches for diagnosing and treating cancer, creating a targeted multifunctional polymer nanoparticle that successfully images and kills brain tumors in laboratory animals (Reddy et al. 2006). The team developed of a 40-nm-diameter polyacrylamide nanoparticle loaded with Photofrin, a photosensitizing agent, and iron oxide. When irradiated with laser light, Photofrin, which is used to treat several types of cancer, including esophageal, bladder, and skin cancers, triggers the production of reactive oxygen species that destroy a wide variety of molecules within a cell. The iron oxide nanoparticles function as an MRI contrast agent. As the targeting agent, the researchers used a 31-amino-acid-long peptide developed by members of the NCI-funded Center of Nanotechnology for Cancer at the University of California (San Diego, CA). This peptide targets an unknown receptor found on the surface of new blood vessels growing around tumors and also triggers cell uptake of nanoparticles attached to it. Researchers tested the nanoparticles in cell cultures and animal models. The studies showed that the nanoparticles traveled to the tumor, resulting in less Photofrin exposure throughout the body, and enhanced exposure within the tumor. This allowed a larger window for activating the drug with light, which was accomplished by threading a fiber optic laser into the brain. In humans, this approach could reduce or eliminate a common side effect of photodynamic therapy, in which healthy skin becomes sensitive to light.

Nanoparticles for Delivery of Drugs to Brain Tumors Across BBB

Nanoparticles may be especially helpful for the treatment of malignant brain tumors. Nanoparticles made of poly(butyl cyanoacrylate) (PBCA) or PLGA coated with polysorbate 80 or poloxamer 188 enable the transport of cytostatics such as doxorubicin across the BBB. Following intravenous injection to rats bearing intracranial glioblastoma, these particles loaded with doxorubicin significantly increased the survival times and led to a complete tumor remission in 20–40 % of the animals (Kreuter and Gelperina 2008). Moreover, these particles considerably reduced the dose-limiting cardiotoxicity and also the testicular toxicity of this drug. The drug transport across the BBB by nanoparticles appears to be due to a receptor-mediated interaction with the brain capillary endothelial cells, which is facilitated by certain plasma apolipoproteins adsorbed by nanoparticles in the blood.

SPION conjugates, prepared using a novel circulating system, have been used to locate brain tumors earlier and more accurately than current methods and to target the tumors (Zhang et al. 2004). A simple dialysis method was developed to immobilize nanoparticles with functional biopolymers and targeting agents, which avoids the use of the normal centrifugation process that may cause particle agglomeration during the coating process. To enhance the specific targeting capability of the nanoparticles, a new chemical scheme was introduced, in which folic acid (FA) was chosen as the targeting agent combined with PEG serving to improve biocompatibility of nanoparticles. AFM characterization showed that the nanoparticles produced are well dispersed with a narrow size distribution. The biological part of the study showed that coating nanoparticles with PEG-FA significantly enhanced the intracellular uptake of nanoparticles by target cells. The researchers plan to attach a variety of small molecules, such as tumor receptor target, and even chemotherapy agents, to the nanoparticles.

MRI can detect the incorporation into brain tumor vasculature of systemically administered bone marrow stem cells labeled with superparamagnetic iron oxide nanoparticles as part of ongoing angiogenesis and neovascularization (Anderson et al. 2005). This technique can be used to directly identify neovasculature in vivo and to facilitate gene therapy by noninvasively monitoring these cells as gene delivery vectors.

A polymeric nanobioconjugate based on biodegradable, nontoxic, and nonimmunogenic polymalic acid as a universal delivery nanoplatform is used for design of a nanomedicine for intravenous treatment of brain tumors (Ding et al. 2010). The polymeric drug passes through the BTB and tumor cell membrane using tandem monoclonal antibodies targeting the BTB and tumor cells. The next step for polymeric drug action is inhibition of tumor angiogenesis by specifically blocking the synthesis of a tumor neovascular trimer protein, laminin-411, by attached antisense oligonucleotides, which are released into the target cell cytoplasm via pH-activated trileucine, an endosomal escape moiety. Introduction of a trileucine endosome escape unit results in significantly increased antisense oligonucleotide delivery to tumor cells, inhibition of laminin-411 synthesis, specific accumulation in brain tumors, and suppression of intracranial glioma growth compared with pH-independent leucine ester. The availability of a systemically active polymeric drug delivery system that crosses BTB, targets tumor cells, and inhibits tumor growth is a promising strategy of glioma treatment.

NP Delivery Across the BBB for Imaging and Therapy of Brain Tumors

In vivo application of nanoparticle-based platforms in brain tumors is limited by insufficient accumulation and retention within tumors due to limited specificity for the target, and an inability to traverse the BBB. A nanoprobe has been designed that can cross the BBB and specifically target brain tumors in a genetically engineered mouse model, by using in vivo magnetic resonance and biophotonic imaging, as well as histologic and biodistribution analyses (Veiseh et al. 2009). The nanoprobe is made of an iron oxide nanoparticle coated with biocompatible PEG-grafted

chitosan copolymer, to which a tumor-targeting agent, chlorotoxin (a small peptide isolated from scorpion venom), and a near-IR fluorophore are conjugated. The particle was about 33 nm in diameter when wet, i.e., about a third the size of similar particles used in other parts of the body. The nanoprobe shows an innocuous toxicity profile and sustained retention in tumors. The nanoparticles remained in mouse tumors for up to 5 days and did not show any evidence of damaging the BBB. With the versatile affinity of the targeting ligand and the flexible conjugation chemistry for alternative diagnostic and therapeutic agents, this nanoparticle platform can be potentially used for the diagnosis and treatment of a variety of brain tumors. The fluorescent nanoparticles improved the contrast between the tumor tissue and the normal tissue in both MRI and optical imaging, which are used during surgery to see the tumor boundary more precisely. Precise imaging of brain tumor margins is important because patient survival for brain tumors is directly related to the amount of tumor that can be resected.

Nano-imaging could also help with early detection of brain tumors. Current imaging techniques have a maximum resolution of 1 mm. Nanoparticles could improve the resolution by a factor of 10 or more, allowing detection of smaller tumors and earlier treatment. Future research will evaluate this nanoparticle's potential for treating tumors.

Intravenous Gene Delivery with Nanoparticles into Brain Tumors

Brain tumors may be amenable to gene therapy with cytotoxic genes, such as the proapoptotic Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand (Apo2L/TRAIL). Gene therapy of gliomas ideally employs intravenously given vectors, thus excluding viral vectors as they cannot cross the BBB. Cationic albumin-conjugated pegylated nanoparticles (CBSA-NP) have been synthesized and shown to accumulate in mouse brain cells upon IV administration. Plasmid pORF-hTRAIL (pDNA) has been incorporated into CBSA-NP, and the resulting CBSA-NP-hTRAIL was evaluated as a nonviral vector for gene therapy of gliomas (Lu et al. 2006). Thirty minutes after IV administration of CBSA-NP-hTRAIL to BALB/c mice bearing intracranial C6 gliomas, CBSA-NP-hTRAIL colocalized with glycoproteins in brain and tumor microvasculature and, via absorptive-mediated transcytosis, accumulated in tumor cells. At 24 and 48 h after intravenous administration of CBSA-NP-hTRAIL, respectively, hTRAIL mRNA and protein were detected in normal brain and tumors. Furthermore, repeated IV injections of CBSA-NP-hTRAIL induced apoptosis in vivo and significantly delayed tumor growth. In conclusion, this study indicates that CBSA-NP-hTRAIL is a promising candidate for noninvasive gene therapy of malignant glioma.

PLA Nanoparticles for Controlled Delivery of BCNU to Brain Tumors

BCNU-loaded biodegradable PLA nanoparticles have been combined with transferrin, an iron-transporting serum glycoprotein, which binds to receptors expressed on surface of glioma cells (Kang et al. 2009). In vitro drug release studies have

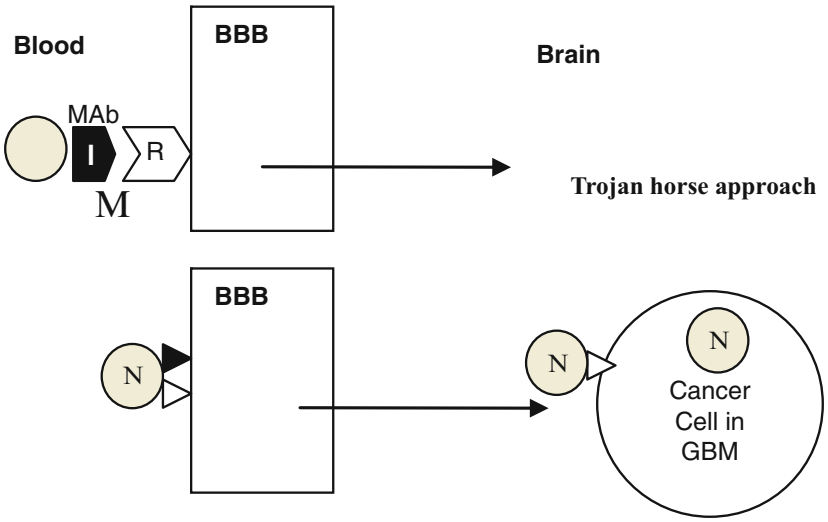

Fig. 9.2 A concept of targeted drug delivery to GBM across the BBB (Nanoparticle (N) combined with a monoclonal antibody (MAb) for receptor (R) crosses the blood brain barrier (BBB) into brain by Trojan horse approach. N with a ligand targeting BBB ▶ traverses the BBB by receptor-mediated transcytosis. Ligand ⊳ docks on a cancer cell receptor and N delivers anticancer payload to the cancer cell in glioblastoma multiforme (GBM). © Jain PharmaBiotech)

demonstrated that BCNU-loaded PLA nanoparticles show certain sustained-release characteristics. The biodistribution of transferrin-coated nanoparticles, investigated by 99Tc-labeled SPECT, showed that the surface-containing transferrin PLA nanoparticles were concentrated in the brain and no radioactive foci could be found outside the brain. Inhibition of tumor growth in the C6 tumor-bearing animal model showed that BCNU-loaded PLA NPs had stronger cytotoxicity and prolonged the average survival time of rats. In contrast to the BCNU wafer approach, the stereotactic method of delivery used in this study may be useful in the development of a new method for delivery of chemotherapy to malignant brain tumors.

NP-Based Targeted Delivery of Chemotherapy Across the BBB

Some of techniques used for facilitating transport of therapeutic substances across the BBB involve damage to the BBB, which is not desirable. Technologies based on nanoparticles targeted delivery of anticancer drugs across the BBB. A concept of targeted drug delivery to GBM across the BBB is shown in Fig. 9.2.

Nanoparticles as Nonviral Vectors for CNS Gene Therapy

Viral vectors for gene delivery to neuronal cells can achieve high transfection efficiency, but problems, such as host immune responses and safety concerns, currently

restrict their use in humans. Nonviral nanoparticles represent a good alternative to viral vectors, but transfection efficiency has to be increased to reach levels that would be relevant for therapeutic purposes.

Silica Nanoparticles for CNS Gene Therapy

Use of organically modified silica (ORMOSIL) nanoparticles (≈30 nm) has been reported as a nonviral vector for efficient in vivo gene delivery without toxic effects and with efficacy equaling or exceeding that obtained in studies using a viral vector (Bharali et al. 2005). Highly monodispersed, stable aqueous suspensions of nanoparticles, surface-functionalized with amino groups for binding of DNA, were prepared and characterized. Intraventricular and intracerebral stereotaxic injections of nanoparticles, complexed with plasmid DNA encoding for EGFP (enhanced green fluorescent protein), were made into the mouse brain. Use of an optical fiber in vivo imaging technique enabled observation of the brain cells expressing genes without having to sacrifice the animal. The ORMOSIL-mediated transfections also were used to manipulate the biology of the neural stem/progenitor cells in vivo. Transfection of a plasmid expressing the nucleus-targeting fibroblast growth factor receptor type 1 resulted in significant inhibition of the in vivo incorporation of bromodeoxyuridine into the DNA of the cells in the subventricular zone and the adjacent rostral migratory stream. Targeted dopamine neurons, which degenerate in Parkinson's disease, take up and express a fluorescent marker gene, demonstrating the ability of nanoparticle technology to effectively deliver genes to specific types of cells in the brain. The gene–nanoparticle complexes were shown to activate adult brain stem/progenitor cells in vivo, which could be effective replacements for those destroyed by neurodegenerative diseases. Thus, ORMOSIL nanoparticles have a potential for effective therapeutic manipulation of the neural stem/progenitor cells as well as in vivo targeted brain therapy. The structure and composition of ORMOSIL allow for the development of an extensive library of tailored nanoparticles to target gene therapies for different tissues and cell types.

Cationic Lipids for CNS Gene Therapy

Cationic lipids show very low transfection efficiency in neurons. They are useful for single-cell studies but not for lack-of-function studies. Generally, cationic lipids are toxic to neurons, although different lipidic formulations can decrease toxicity. They are not effective for gene delivery to the brain when administered intravenously, although "Trojan horse" liposomes can be an exception.

Polyethylenimine-Based Nanoparticles for CNS Gene Therapy

Polyethylenimine (PEI) nanoparticles have higher transfection efficiency (20 %) than cationic lipids in neurons, but this is still very low for therapeutic purposes. At least 70–80 % transfection efficacy is required for removal of a protein.

These nanoparticles are toxic for neurons, and modifications of the molecule, such as PEGylation, are required to decrease neurotoxicity. Moreover, PEI-based nanoparticles are only effective for gene delivery when injected locally, which precludes their development for clinical use at present.

Dendrimers for CNS Gene Therapy

Dendrimers are capable of very efficient neuronal transfection in vitro (transfection efficiencies of 75 % have been achieved) with low toxicity when external amino groups are masked by surface functionalization. Further developments need to be carried out to enable efficient BBB crossing, in order to deliver genetic material to neurons and glial cells. Dendrimers are the most promising particles for genetic material delivery to the CNS either alone or in combination with carbon-based nanoparticles (nanotubes and nanohorns).

Carbon Nanotubes for CNS Gene Therapy

CNTs avoid endosomes and, once functionalized, their solubility is increased to make them biocompatible and capable of delivery of genetic material to different cells. Coupled to dendrimers, CNTs represent a new concept that can play a relevant role in gene therapy in the nervous system, if toxicological issues are solved (Posadas et al. 2010). Once the safety has been established, CNT-based vectors should be able to perform an "enhanced" gene transfer in target cells. Potential applications include cerebral ischemia and Rett syndrome.

Nanoparticle-Based Drug Delivery to the Inner Ear

Drug delivery to the inner ear is important for the treatment of inner ear disorders such as those involving hearing. Another disorder, tinnitus, is a problem in management and several innovative approaches are under investigation. An obstacle to effective treatment of inner ear diseases is the atraumatic delivery of therapeutics into inner ear perilymph. It is feasible to use SPIONs as drug delivery vehicles. As a minimally invasive approach, intratympanic delivery of multifunctional nanoparticles (MFNPs) carrying genes or drugs to the inner ear is a future therapy for treating inner ear diseases, including sensorineural hearing loss (SNHL) and Meniere's disease. Liposome nanoparticles encapsulating gadolinium-tetra-azacyclo-dodecane-tetra-acetic acid (LPS + Gd-DOTA) are visible by MRI in the inner ear in vivo after either intratympanic or intracochlear administration demonstrating transport from the middle ear to the inner ear and their distribution in the inner ear (Zou et al. 2010). Passive diffusion of fluorescent NPs through the round window membrane (RWM) within the freshly frozen human temporal bone has been demonstrated, and these NPs were subsequently found to be distributed in the sensory hair cells, nerve fibers, and to other cells of the cochlea (Roy et al. 2012). Nontoxic NPs have a great potential for controlled drug delivery to the human inner ear across the RWM.

Nanotechnology-Based Devices and Implants for CNS

Nanoparticle-mediated drug delivery to the brain, as described in previous sections, will minimize the need for use of invasive delivery devices, but there will still be need for implants and direct delivery of drugs to the brain and the cerebral ventricles. Nanomaterials, because of their action in preventing the formation of scar due to astrocyte proliferation, would improve the construction of nonreactive cerebroventricular catheters for administration of drugs into the cerebral ventricles. Nanoengineered probes can deliver drugs at the cellular level using nanofluidic channels.

Nanobiotechnology and Neuroprotection

Nanoparticles can improve drug delivery to the CNS and facilitate crossing of BBB and more precisely target a CNS injury site. These technologies were described in Chap. 6 and the topic of neuroprotection is dealt with in detail in a handbook on this topic (Jain 2011). Some nanoparticles have a neuroprotective effect.

QD technology has been used to gather information about how the CNS environment becomes inhospitable to neuronal regeneration following injury or degenerative events by studying the process of reactive gliosis. Other research is looking at how QDs might spur growth of neurites by adding bioactive molecules to the QDs, in a way to provide a medium that will encourage this growth in a directed way. PLGA nanoparticles loaded with superoxide dismutase have neuroprotective effect seen up to 6 h after H_2O_2-induced oxidative stress, which appears to be due to the stability of the encapsulated enzyme and its better neuronal uptake after encapsulation (Reddy et al. 2008).

Gold salts, known to have an immunosuppressive effect, have been considered for treatment of TBI, which results in loss of neurons caused not only by the initial injury but also by the resulting neuroinflammation as a secondary effect. The systemic use of gold salts is limited by nephrotoxicity. However, implants of pure metallic gold release gold ions, which do not spread in the body but are taken up by cells near the implant. This is a safer method of using to reduce local neuroinflammation. Release or dissolucytosis of gold ions from metallic gold surfaces requires the presence of disolycytes, i.e., macrophages, and the process is limited by their number and activity. In one study, the investigators injected 20–45-μm gold particles into the neocortex of mice before generating a cryo-injury (Larsen et al. 2008). Comparison of gold-treated and untreated cryolesions showed that the release of gold reduced microgliosis and neuronal apoptosis accompanied by a transient astrogliosis and an increased neural stem cell response indicating anti-inflammatory and neuroprotective effect. Intracerebral application of metallic gold as a pharmaceutical source of gold ions bypasses the BBB and enables direct drug delivery to inflamed brain tissue. The method of delivery is invasive and a gold implant could produce foreign body reaction leading to an epileptic focus. This can be refined by the use of gold nanoparticles.

Cadmium telluride (CdTe) nanoparticles (NPs) can efficiently prevent amyloid beta (Aβ) fibril formation, a pathological feature of Alzheimer's disease (AD), based on the multiple binding of Aβ oligomers to CdTe NPs (Yoo et al. 2011). By introducing tetrahedral CdTe NPs that were comparable in size with growing fibrils, the researchers discovered that the Aβ plaque readily bonded to them and CdTe NP geometry was strongly distorted resulting in complete inhibition of further growth of Aβ fibrils. CdTe NPs can inhibit the Aβ fibril formation in minute quantities with much greater efficiency; 1 CdTe NP can capture more than 100 amyloid peptides. This high efficiency of CdTe NPs is similar to some proteins that the human body uses to prevent formation of Aβ fibrils and protect itself against the progression of AD. These findings provide new opportunities for the development of drugs to prevent AD.

Nanobiotechnology for Regeneration and Repair of the CNS

Nanobiotechnology applications, aimed at the regeneration and neuroprotection of the CNS, will significantly benefit from basic nanotechnology research conducted in parallel with advances in cell biology, neurophysiology, and neuropathology. The aim is to help neuroscientists better understand the physiology of and develop treatments for disorders such as traumatic brain injury (TBI), spinal cord injury (SCI), degenerative retinal disorders, and neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD).

Nanowire Neuroprosthetics with Functional Membrane Proteins

Living organisms use a sophisticated arsenal of membrane receptors, channels, and pumps to control signal transduction to a degree that is unmatched by man-made devices. Electronic circuits that use such biological components could achieve drastically increased functionality; however, this approach requires nearly seamless integration of biological and man-made structures. A versatile hybrid platform for such integration has been constructed that uses shielded nanowires coated with a continuous lipid bilayer (Misra et al. 2009). When shielded silicon nanowire transistors incorporate transmembrane peptide pores gramicidin A and alamethicin in the lipid bilayer, they can achieve ionic to electronic signal transduction by using voltage-gated or chemically gated ion transport through the membrane pores. The membrane pore could be opened and closed by changing the gate voltage of the device to enable monitor specific transport and also to control the membrane protein. The work shows promise for enhancing biosensing and diagnostics tools, and neural prosthetics such as cochlear implants.

Nanotube–Neuron Electronic Interface

Thin films of CNTs deposited on transparent plastic can also serve as a surface on which cells can grow, and these nanotube films could potentially serve as an electrical interface between living tissue and prosthetic devices or biomedical instruments. University of Texas Medical Branch's scientists have shown that there is some kind of electrical communication between these two things, by stimulating cells through the transparent conductive layer (Liopo et al. 2006). The scientists employed two different types of cells in their experiments, neuroblastoma cells commonly used in test-tube experiments and neurons cultured from experimental rats. Both cell types were placed on ten-layer-thick "mats" of SWCNTs deposited on transparent plastic. This enabled the researchers to use a microscope to position a tiny electrode next to individual cells and record their responses to electrical pulses transmitted through the SWCNTs. In addition to their electrical stimulation experiments, the scientists also studied how different kinds of SWCNTs affected the growth and development of neuroblastoma cells. They compared cells placed on mats made of "functionalized" SWCNTs, carbon nanotubes with additional molecules attached to their surfaces that may be used to guide cell growth or customize nanotube electrical properties, to cells cultured on unmodified "native" carbon nanotubes and conventional tissue culture plastic. Native CNTs supported neuron attachment and growth well better than the two types of functionalized nanotubes tested. Next step in the research is to find a way to functionalize the nanotubes to make neuron attachment and communication better and make these surfaces more biocompatible. If nanotubes turn out to be sensitive enough to record ongoing electrical activity in cells, they could form the basis of a device that can both sense and deliver stimuli to cells for prosthetic control.

Role of Nanobiotechnology in Regeneration and Repair Following CNS Trauma

Repair and regeneration following CNS trauma requires a multifaceted approach (Jain 2012g). Role of nanobiotechnology in various strategies for regeneration and repair following CNS trauma are listed in Table 9.1.

Nanofibers as an Aid to CNS Regeneration by Neural Progenitor Cells

One approach to growing nerve cells in tissue cultures is to encapsulate neural progenitor cells in vitro within a 3D network of nanofibers formed by self-assembly of peptide amphiphile molecules. The self-assembly of nanofiber scaffold is initiated by mixing cell suspensions in media with dilute aqueous solutions of the molecules, and cells survive the growth of the nanofibers around them. These nanofibers are designed to present to cells the neurite-promoting laminin epitope. Relative to laminin

Table 9.1 Role of nanobiotechnology in regeneration and repair following CNS trauma

Neuroprotective nanoparticles to prevent further damage
Nanofibers to provide scaffolds for regeneration and reducing or eliminating scar formation
Nanofibers for providing cues to axons for regeneration
Nanoparticles for repair of injured neurons and nerve fibers by sealing them
Nanoparticles to track stem cells implanted to replace the loss and to promote growth of neural tissues
Nanoparticle-based delivery of drugs to promote growth of neural tissues

or soluble peptide, the artificial nanofiber scaffold induces very rapid differentiation of neural progenitor cells into neurons, while discouraging the development of astrocytes.

These new materials, because of their chemical structure, interact with cells of the CNS in ways that may help prevent the formation of scar due to astrocyte proliferation that is often linked to paralysis resulting from traumatic spinal cord injury (SCI). Silicon neural electrodes are engineered with a nanostructured form of silicon called porous silicon, which acts as a scaffold that reduces glial scarring from electrode implantation and enhances neural growth at the brain recording sites to create a superior interface with neurons. This would be useful in the procedure of electrode implantation in neurological disorders such as PD and epilepsy.

Peptide Nanostructures for Repair of the CNS

Peptide nanostructures containing bioactive signals offer novel therapies with potential impact on regenerative medicine. These nanostructures can be designed through self-assembly strategies and supramolecular chemistry and can combine bioactivity for multiple targets with biocompatibility. It is also possible to multiplex their functions by using them to deliver proteins, nucleic acids, drugs, and cells. Self-assembling peptide nanostructures can facilitate regeneration of the CNS. Other self-assembling oligopeptide technologies and the progress made with these materials toward the development of potential therapies have been reviewed elsewhere (Webber et al. 2010).

Nanobiotechnology for Repair and Regeneration Following TBI

Challenges of using a tissue engineering approach for regeneration in TBI include a complex environment and variables that are difficult to assess. For optimal benefit, the brain should be in a condition that minimizes immune response, inflammation, and rejection of the grafted material. Tissue engineering, using a bioactive scaffold, counters some of the hostile factors and facilitates integration of donor cells into the brain, but transplantation of a combination biologic construct to the brain has not yet been successfully translated into clinical use (Stabenfeldt et al. 2011).

The next generation of tissue engineering scaffolds for TBI may incorporate nanoscale surface feature dimensions, which mimic natural neural tissue. Nanomaterials can enhance desirable neural cell activity while minimizing unwanted astrocyte reactivity. Composite materials with zinc oxide nanoparticles embedded into a polymer matrix can provide an electrical stimulus when mechanically deformed through ultrasound, which can act as a cue for neural tissue regeneration (Seil and Webster 2010).

Nanoparticles for Repair Following SCI

SCI can lead to serious neurological disability, and the most serious form of it is paraplegia or quadriplegia. Currently, over 250,000 persons in the USA and several millions worldwide are living with permanent disability due to chronic SCI. There are approximately 12,000 new cases of acute SCI in the USA each year. Over 90 % of acute SCI victims now survive their injuries and go on to become part of the chronic SCI population, living paralyzed for an average of more than 40 years after injury.

Local spinal cord lesions are often greatly enlarged by secondary damage, which is accompanied by additional massive cell death that involves neurons, microglia, and macroglia and is virtually complete at 12 h. Immediate care involves stabilization of the patient's general condition by supportive measures. Surgery is carried out in some cases for removal of compressing lesions and stabilization of spinal fractures. A number of neuroprotective strategies are under investigation. Stem cell therapies are also under investigation for neuroregeneration, and nanoparticles can be used to track the course of stem cells. There is no therapeutic measure available currently that enhances functional recovery significantly.

Nanomaterials injected into the severed spinal cords of mice enable them to walk again after several weeks of therapy. The nanomaterials used in these studies were designed to self-assemble into nanofibers, which provide the framework for regeneration of nerve fibers. In a nanofiber network, progenitor cells develop into neurons and not astrocytes that form scar tissue and hinder regeneration. The research offers new insights into the near-term research potential of nanotechnology and offers hope for patients with severe neuron damage due to other causes as well.

Repair of SCI by Nanoscale Micelles

Another key approach for repairing injured spinal cord is to seal the damaged membranes at an early stage. Axonal membranes injured by compression can be effectively repaired using self-assembled monomethoxy PEG–PLA di-block copolymer micelles (Shi et al. 2010). The micelles might be used instead of conventional PEG. A critical feature of micelles is that they combine two types of polymers, one being hydrophobic and the other hydrophilic, meaning they are either unable or able to mix with water. Because of the nanoscale size and the PEG shell of the micelles, they are not quickly filtered by the kidney or captured by the liver, enabling them to remain in the bloodstream long enough to circulate to damaged tissues.

Injured spinal tissue incubated with micelles (60 nm diameter) showed rapid restoration of compound action potential and reduced calcium influx into axons for micelle concentrations much lower than the concentrations of PEG, approximately 1/100,000, for early-stage SCI. Intravenously injected micelles effectively recovered locomotor function and reduced the volume and inflammatory response of the lesion in injured rats, without any adverse effects. These results show that copolymer micelles can interrupt the spread of primary SCI damage with minimal toxicity. The research also showed that without the micelles treatment, about 18 % of axons recover in a segment of damaged spinal cord tested, whereas the micelles treatment boosted the axon recovery to about 60 %. The researchers used the chamber to study how well micelles repaired damaged nerve cells by measuring the "compound action potential," or the ability of a spinal cord to transmit signals.

The experiment mimics what happens during a traumatic SCI. Findings showed that micelles might be used to repair axon membranes damaged by compression injuries, a common type of spine injury. Dyed micelles were also tracked in rats, demonstrating that the nanoparticles were successfully delivered to injury sites. Findings also showed micelles-treated animals recovered the coordinated control of all four limbs, whereas animals treated with conventional PEG did not. Further research will include work to learn about the specific mechanisms that enable the micelles to restore function to damaged nerve cells.

Nanobiotechnology-Based Devices for Restoration of Neural Function

The remarkable optical and electrical properties of nanostructured materials are now considered to be a source for a variety of biomaterials, biosensing, and cell interface applications. Some of the characteristics of nanoparticles can be exploited to custom-build new materials from the bottom up with characteristics such as compatibility with living cells and the ability to turn light into tiny electrical currents that can produce responses in nerves. A study reports construction of a hybrid bionanodevice where absorption of light by thin films of quantum-confined semiconductor nanoparticles of HgTe produced by the layer-by-layer assembly stimulate adherent neural cells via a sequence of photochemical and charge-transfer reactions (Pappas et al. 2007). The development opens the door to applying the unique properties of nanoparticles to a wide variety of light-stimulated nerve-signaling devices including the possible development of a nanoparticle-based artificial retina.

Nanobiotechnology-Based Artificial Retina

Although light signals have previously been transmitted to nerve cells using silicon (whose ability to turn light into electricity is employed in solar cells and in the imaging sensors of video cameras), nanoengineered materials promise far greater efficiency and versatility. It should be possible to tune the electrical characteristics of these nanoparticle films to get properties like color sensitivity and differential

stimulation, which are needed for an artificial retina. Creation of an actual implantable artificial retina is, however, a long-range project. But, a variety of less complex applications are enabled by a tiny, versatile light-activated interface with nerve cells, e.g., ways to connect with artificial limbs and new tools for imaging, diagnosis, and therapy. The main advantage of this technology is that remote activation by light is possible without cumbersome wire connections. This type of technology can provide noninvasive connections between the human nervous system and prostheses that are flexible, compact, and reliable. Such tools will provide nanoneurology new capabilities that were not possible with conventional methods.

Nanoneurosurgery

Neurosurgery is an extension of neurology involving surgery, nanodiagnostics, and application of new technologies for treatment of neurological disorders. Advances in nanobiotechnology have already refined many surgical approaches to diseases of the nervous system, and this new field can be called nanoneurosurgery. Examples are applications in brain cancer, neuroregeneration, and CNS implants.

Femtolaser Neurosurgery

Understanding how nerves regenerate is an important step toward developing treatments for human neurological disease, but investigation has so far been limited to complex organisms (mouse and zebrafish) in the absence of precision techniques for severing axons (axotomy). Femtosecond laser surgery has been used for axotomy in the roundworm *Caenorhabditis elegans*, and these axons functionally regenerated after the operation (Yanik et al. 2004). Femtolaser acts like a pair of tiny "nano-scissors," which is able to cut nanosized structures like nerve axons

The pulse has a very short length making the photons in the laser concentrate in one area, delivering a lot of power to a tiny, specific volume without damaging surrounding tissue. Once cut, the axons vaporize and no other tissue is harmed. The researchers cut axons they knew would impair the worms' backward motion. The worms could not move backward after surgery. But within 24 h, most of the severed axons regenerated and the worms recovered backward movement, confirming that laser's cut did not damage surrounding tissue and allowed the neurons to grow a new axon to reach the muscle. Application of this precise surgical technique should enable nerve regeneration to be studied in vivo.

Nanofiber Brain Implants

Several brain probes and implants are used in neurosurgery. Examples are those for the management of epilepsy, movement disorders, and pain. Many of these implants are still investigational. The ideal inert material for such implants has not yet been

discovered. Silicon probes are commonly used for recording of electrical impulses and for brain stimulation. The body generally regards these materials as foreign, and the probes get encapsulated with glial scar tissue, which prevents them from making good contact with the brain tissue.

An in vitro study was done to determine cytocompatibility properties of formulations containing carbon nanofibers pertinent to neural implant applications (McKenzie et al. 2004). Substrates were prepared from four different types of carbon fibers, two with nanoscale diameters (nanophase, or less than or equal to 100 nm) and two with conventional diameters (or greater than 100 nm). Within these two categories, both a high and a low surface energy fiber were investigated and tested. Astrocytes (glial scar tissue-forming cells) were seeded onto the substrates for adhesion, proliferation, and long-term function studies (such as total intracellular protein and alkaline phosphatase activity). Results provided the first evidence that astrocytes preferentially adhered and proliferated on carbon fibers that had the largest diameter and the lowest surface energy. Formulations containing carbon fibers in the nanometer regime limited astrocyte functions leading to decreased glial scar tissue formation. Positive interactions with neurons and, at the same time, limited astrocyte functions leading to decreased gliotic scar tissue formation are essential for increased neuronal implant efficacy. Nanotubes, because of the interesting electronic properties and reduction in scar formation, hold great promise for replacing conventional silicone implants.

Nanoparticles as an Aid to Neurosurgery

A research team from Oregon Health and Science University (Portland OR) has shown that an iron oxide nanoparticle can outline not only brain tumors under MRI but also other lesions in the brain that may otherwise have gone unnoticed (Neuwelt et al. 2004). Ferumoxtran-10 (Combidex®, AMAG Pharmaceuticals Inc.), a dextran-coated iron oxide nanoparticle, provides enhancement of intracranial tumors by MRI for more than 24 h and can be imaged histologically by iron staining. Each iron oxide nanoparticle is the size of a small virus and is much smaller than a bacterium but much larger than an atom or standard gadolinium contrast molecule. It is an iron oxide crystal surrounded with a carbohydrate or "sugar" coating called dextran, which gives the particle a longer plasma half-life, allowing it to slowly slip through the BBB. Ferumoxtran-10 can also provide a "stable imaging marker" during surgery to remove brain tumors, and it remains in the brain long enough for postoperative MRI, even after surgical manipulation. These findings have the potential to assist image-guided brain surgery and improve diagnosis of lesions caused by multiple sclerosis, stroke, and other neurological disorders, in addition to residual tumors. Because ferumoxtran-10 can stay in brain lesions for days, it can be administered to patients 24 h before surgery and can image other, noncancerous lesions. It has some advantages over gadolinium, a metal used as an MRI contrast agent for 20 years and which must be administered just before surgery. However, it will complement gadolinium but not replace it. Ferumoxtran-10 gives additional information that cannot be obtained in some patients with gadolinium. Using both

the contrast agents, one can get better diagnostic information that has the potential to improve the patient's outcome. In addition, ferumoxtran-10 can be detected with an iron stain in the tissue removed by biopsy or surgery, allowing physicians to see it in brain tissue samples under a microscope. Unlike any other MRI contrast agent, ferumoxtran-10 enables the comparison of images from an MRI scan with the tissue taken out at surgery. Moreover, it is relatively safe when diluted and administered as an infusion.

Nanoscaffold for CNS Repair

There are several barriers that must be overcome to achieve axonal regeneration after injury in the CNS: (1) scar tissue formation, (2) gaps in nervous tissue formed during phagocytosis of dying cells after injury, (3) factors that inhibit axon growth in the mature mammalian CNS, and (4) failure of many adult neurons to initiate axonal extension.

Using the mammalian visual system as a model, a self-assembling peptide nanofiber scaffold was designed, which creates a permissive environment for axons not only to regenerate through the site of an acute injury but also to knit the brain tissue together. In experiments using a severed optic tract in the hamster, it was shown that regenerated axons reconnect to target tissues with sufficient density to promote functional return of vision, as evidenced by visually elicited orienting behavior (Ellis-Behnke et al. 2006). The peptide nanofiber scaffold not only represents a previously undiscovered nanobiomedical technology for tissue repair and restoration but also raises the possibility of effective treatment of CNS and other tissue or organ trauma. This peptide nanofiber scaffold has several advantages over currently available polymer biomaterials: (1) it forms a network of nanofibers that are similar in scale to the native extracellular matrix and therefore provides an "in vivo" environment for cell growth, migration, and differentiation; (2) it can be broken down into natural L-amino acids and metabolized by the surrounding tissue; (3) it is synthetic and free of chemical and biological contaminants that may be present in animal-derived biomaterials such as collagens; and (4) it appears to be immunologically inert, thus avoiding the problem of neural tissue rejection.

Electrospun Nanofiber Tubes for Regeneration of Peripheral Nerves

Several neural prostheses have been used to replace the loss of nervous tissue in peripheral nerve injuries by providing a path for regenerating nerve fibers. Most of these use rigid channel guides that may cause cell loss due to the lack of physiological local stresses exerted over the nervous tissue during the patient's movement. The electrospinning technique makes it possible to spin nanofiber flexible tubular scaffolds, with high porosity and surface/volume ratio. Electrospun tubes made of

biodegradable polymers (a blend of PLGA/PCL) have been used to regenerate a 10-mm nerve gap in a rat sciatic nerve (Panseri et al. 2008). In most of the treated animals, the electrospun tubes induced neural regeneration and functional reconnection of the two severed sciatic nerve tracts. Myelination occurred and no significant inflammatory responses were observed. Reestablishment of functional neuronal connections with reinnervation of the affected muscles was demonstrated by neural tracers and evoked potential recordings. These findings show that electrospun tubes, with additional biological coating or incorporated drugs, are promising scaffolds for functional neural regeneration. They can be knitted in meshes, and their mechanical properties can be tuned to provide biomimetic functionalization. Moreover, the conduits can be loaded with neurotrophic factors and seeded with stem cells.

Buckyballs for Brain Cancer

Buckyballs (fullerenes) are under investigation to improve the ability of MRIs to locate brain tumors and deliver a payload of radiation to destroy them. Experiments on rats have shown that buckyballs packed with the MRI contrast metal gadolinium can increase the sensitivity of MRI detection by at least 40-fold. This level of precision is reaching a point at which cancer cells that have spread beyond the margins of the tumor may become visible. Stray cells, left behind after surgery, are thought to be responsible for tumor relapse. Finding and removing these cells could improve a patient's chance of survival. The scientists have created a modified version of the buckyballs with a fluorescent metal atom called terbium, which could guide surgeons to remove tumors with greater precision. Addition of yet another metal, lutetium, would deliver a lethal dose of radiation to the cancer cells, including those missed by the surgeon. The research is a few years away from testing in humans, but the potential is promising.

Application of Nanobiotechnology to Pain Therapeutics

Nanotechnology offers the potential to address multiple, major unmet problems in the diagnosis, treatment, and symptom management of a large variety of diseases and conditions, including cancer. Nanobiotechnology will contribute to improvement of cancer pain therapeutics through facilitation of drug discovery for pain. A more immediate application is in facilitating drug delivery for pain. A transbuccal transmucosal system, Buccal Patch®, has been developed for the administration of remifentanil for the management of breakthrough cancer pain (Sprintz et al. 2005). The nanochannel size of the device permits the diffusion of the drug from its reservoir to the target tissue at a consistent and controlled rate, minimizing the risk of overdosing the patient. Intravenous administration of ibuprofen in lipid nanocapsules formulation has an advantage as an analgesic over oral preparations.

US Army is supporting research to develop nanoparticle-based analgesics that can be injected with a pen-like device by injured soldiers' comrades, or even injured soldiers themselves, on the battlefield. The method will use analgesic drugs coupled to polymers but can be released to provide adequate pain relief as well as antidotes to avoid adverse effects of these drugs. For example, morphine, an analgesic commonly used to treat wounded soldiers, needs to be injected by skilled medical personnel. Patients who receive morphine need to be monitored carefully because the painkiller can cause breathing problems. These requirements restrict the use of morphine on the battlefield. If successful, the nanotechnology approach could markedly improve the treatment of soldiers in the field. Various types of nanoparticles will be designed and tested. The aim is to create nanoparticles that can achieve the following objectives:

- Control the release of morphine over extended periods to ensure pain relief until a soldier can be evacuated to a military acute care facility
- Continuously monitor the soldier's breathing and, if needed, release the drug naloxone, which counters morphine's effects on breathing