Chapter 14

Cell Culture Techniques

After Reading This Chapter, You Should be Able to:

- Compare the advantages and disadvantages of using *in vitro* culture techniques with *in vivo* techniques
- Describe types of cells used to examine nervous system function *in vitro*
- Describe techniques for manipulating cells in cell culture conditions

Techniques covered:

- **Tools and reagents used in culture**: equipment, media
- **Types of cultured cells:** immortalized cell lines, primary cell and tissue culture, stem cells
- **Manipulating cells in culture:** transfection, co-culture, pharmacology, antibody interference

Cell culture is the process by which a scientist grows and maintains cells under carefully controlled conditions outside of a living animal. There are many reasons why this approach is desirable. Examining the nervous system *in vitro* (within glass) allows scientists to simplify the cellular environment, providing greater control over experimental manipulations and reducing potentially confounding interactions with other biological systems. *In vitro* tools and techniques make experiments possible that would otherwise be difficult or impossible to perform (or interpret) in intact organisms, such as performing multiple assays in parallel with the exact same number of cells. *In vitro* experiments also tend to be faster, less expensive, and require fewer animals than experiments performed *in vivo*.

However, cell culture experiments also raise interesting questions: Does a cell in a culture flask truly behave like it would inside the brain or body? What does it mean to culture neurons, cells notorious for their intercellular communication and neural networks, in relatively isolated conditions in which they do not form synaptic connections? Are immortalized cell lines, cells that continue to divide indefinitely, actually good models for cells that degenerate and perish in the brain? Because the goal of most *in vitro* experiments is to generate a hypothesis and conclusion about what occurs *in vivo*, it is important to design experiments carefully and extrapolate results cautiously.

This chapter surveys approaches to studying the nervous system using cell culture techniques. We start with a quick summary of the equipment and

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reagents necessary to maintain cells outside their endogenous environments. Then we describe three categories of cells used in culture experiments: immortalized cell lines, primary cell cultures, and a special class of cell that has received much attention over the past several years—stem cells. Finally, we describe some of the methods scientists can use to perturb cells in cultured environments.

CELL CULTURE EQUIPMENT AND REAGENTS

Specialized equipment and reagents are necessary to provide cultured cells with an environment that can support their continued growth and health outside a living organism. Most of these supplies are used to artificially mimic the endogenous, *in vivo*, cellular environment. Taking a cell out of the brain and expecting it to survive in a cell culture dish without providing basic, life-sustaining factors would be like catching a fish and expecting it to stay alive in a cage: unless a scientist correctly provides the elements necessary to sustain life, the cell will quickly die. Thus, most equipment and reagents in cell culture labs are used to continually supply cells with oxygen, nutrients, growth factors, and other elements necessary to keep cells alive.

Other tools and reagents prevent contamination. Cells *in vivo* have the benefit of an active immune system to prevent contamination from bacteria, fungi, and other microorganisms. Cells in the brain have the added benefit of the blood–brain barrier to further prevent contamination. In culture conditions, cells are incredibly vulnerable, and scientists must take great care in avoiding contamination, especially because microorganisms are ubiquitous in the environment and can easily penetrate cell culture plates.

Equipment

Although individual laboratories study different cell lines and ask different scientific questions, cell culture rooms tend to contain the same fundamental pieces of equipment. This equipment includes the following:

- *Biosafety hood.* A biosafety hood or laminar flow cabinet is used to prevent contamination by microorganisms (Figure 14.1). When not in use, these chambers are often illuminated with UV light that helps sterilize exposed surfaces. Just before use and throughout experiments, scientists spray all surfaces with 70% ethanol to provide further decontamination. During experiments, scientists always keep reagents and cell culture flasks/plates covered until they are ready for use; when exposed, a scientist should never pass their gloved hands or other equipment over open bottles or, even worse, the cells themselves.
- *Cell incubator.* Cell incubators house and store culture flasks and plates. These incubators maintain an appropriate temperature, humidity, and gas concentration to mimic endogenous conditions. They are usually set at 37 °C with 5% CO_2 levels. Although 5% CO_2 is not the level experienced in the body *in vivo*, the carbon dioxide maintains the pH of buffers in the growth media at proper physiological levels.

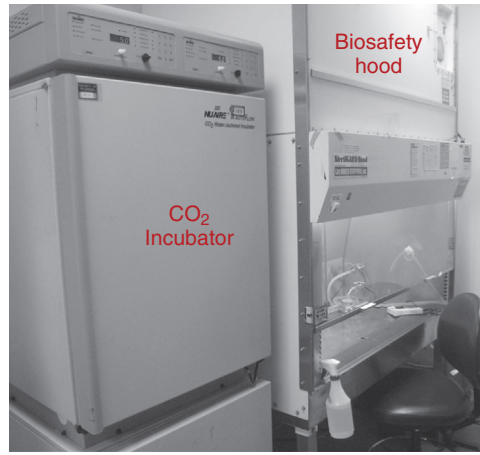

FIGURE 14.1 **Typical cell culture room with biosafety hood and CO_2 incubator.**

- *Specially prepared cell culture flasks/plates.* Tissue culture flasks and plates come in many varieties depending on the needs of the investigator. Flasks typically range in sizes of 12.5 cm^2 to large, multitiered chambers with surface areas of 1800 cm^2. Multiwelled plates are often used for tissue culture experiments, as these wells can hold an exact number of cells, and it is easy to keep track of various experimental conditions across wells. Plates are typically sold with 6, 12, 24, 48, 96, or 384 wells. Many cells, including neurons, need to adhere to a substrate to grow, so tissue culture dishes often need to be specially prepared to provide a substrate for the cells. Tissue culture dishes or glass coverslips are often coated with attractive amino acids, such as lysine or ornithine or extracellular matrix components like collagen and laminin.
- *Refrigerator.* A refrigerator (properly referred to as a 4 °C incubator) maintains cell culture media and other reagents when not in use. The growth factors and antibiotics in culture media degrade over time, but can last for weeks if stored at 4 °C.
- *Water bath.* A water bath is often set at 37 °C and is used to quickly warm cell culture media and other reagents stored at 4 °C just prior to being added to cell culture flasks/plates. If a scientist does not warm media before adding it to cells, the cells can be shocked by the abrupt change to cold temperatures and die.
- *Microscope.* Microscopes are used in most tissue culture rooms for routine observation of cell culture flasks/plates to inspect the health and confluence of cells. **Confluence** refers to the percentage of the surface of the bottom of the plate covered by cells. Most cell lines should never become 100% confluent, as cells in dense populations tend to inhibit each other's growth, as well as quickly drain nutrients from the culture media.

Culture Media

Growth media is critical to cell culture experiments, supplying nutrients (amino acids and vitamins) and a source of energy (glucose) for cells. Growth media can vary in pH, nutrient concentration, and the presence of growth factors or other biologically relevant components. To survive, cells must be bathed in an isotonic fluid that has the same concentration of solute molecules as inside the cell. The media is buffered to maintain a compatible pH (usually 7.4, though there are some cell-specific variations). In the 5% CO_2 environment of culture incubators, a bicarbonate buffer maintains the physiological pH as well as providing nutritional benefits to the cells. HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) is added to the culture media for extra buffering capacity when the cells in a culture experiment require extended periods of manipulation outside a CO_2 incubator.

Serum is often added to culture media for its ability to promote survival through undefined mixtures of growth factors, hormones, and proteins, like platelet-derived growth factor, insulin, and transferrin. For stricter control over the cellular environment, investigators use serum-free, chemically defined supplements, such as N2 or B27, that contain known formulations of survival factors. A typical growth medium in neuronal cultures is NeuroBasal, which provides optimized amino acids and nutrients to cultured neurons, supplemented with N2 or B27.

Adding or removing specific ingredients to or from the media can influence cellular behavior. For example, to maintain a neural progenitor pool that continues to divide in culture, scientists add growth factors such as epidermal growth factor (EGF) and fibroblast growth factor (FGF) to prevent differentiation. Specific recipes for culture media are tailored to provide optimal conditions for promoting the health and proper physiological function of the cultured cells and for maintaining cellular behavior.

Now that we have described some of the equipment and reagents necessary for cell culture, we survey the different categories of cells commonly used in cell culture experiments.

IMMORTALIZED CELL LINES

Immortalized cell lines are either tumorous cells that do not stop dividing or cells that have been artificially manipulated to proliferate indefinitely and can, thus, be cultured over several generations (Table 14.1). Because immortalized cells continuously divide, they eventually fill up the dish or flask in which they grow. By **passaging** (also known as **splitting**), scientists transfer a fraction of the multiplying cells into new dishes to provide space for continuing proliferation.

There are many advantages to using immortalized cell lines. Because these are standard lines used by many different labs, immortalized cells are fairly well characterized. They are, at least theoretically, homogeneous, genetically

TABLE 14.1 Commonly Used Immortalized Cell Lines

	Origin and cell type	Comments
3T3	Mouse embryonic fibroblast	Robust and easy to handle; contact inhibited; stops growing at very high densities
HeLa	Human epithelial cell	From cervical cancer in a human patient named Henrietta Lacks; may contaminate other cultured cell lines; able to grow in suspension (i.e., grow without adhering to bottom of plates)
COS (**C**v-1 in **O**rigin, carrying **S**v40 genetic material)	Monkey kidney	Efficiently transfected; commonly used as an expression system for high-level, short-term expression of proteins
293/293T/HEK-293T	Human embryonic kidney	Easy to transfect and manipulate; commonly used as an expression system to study signaling and recombinant proteins
MDCK (Madin-Darby canine kidney)	Dog kidney epithelial cell	Polarized with distinct apical and basal sides, used in studying trafficking
CHO	Chinese hamster ovary	Useful for stable gene expression and high protein yields for biochemical assays; commonly used as an expression system for studying cell signaling and recombinant proteins
S2	*Drosophila* macrophage-like cells	Well-characterized *Drosophila* cell line; highly susceptible to RNAi treatment
PC12	Rat pheochromocytoma chromaffin cell	Neuron-like, derived from a neuroendocrine adrenal tumor; can differentiate into a neuron-like cell in the presence of nerve growth factor

Continued

TABLE 14.1 Commonly Used Immortalized Cell Lines—cont'd

	Origin and cell type	Comments
Neuro-2a/N2a	Mouse neuroblastoma	Model system for studying pathways involved in neuronal differentiation; can be driven to differentiate by cannabinoid and serotonin receptor stimulation
SH-SY5Y	Human neuroblastoma, cloned from bone marrow	Dopamine beta hydroxylase activity, acetylcholinergic, glutamatergic, adenosinergic; grow as clusters of neuroblast-type cells with short, fine neurites

identical populations, which aid in providing consistent and reproducible results. Immortalized cells tend to be easier to culture than cells used in primary cultures in that they grow more robustly and do not require extraction from a living animal. Also, because they grow quickly and continuously, it is possible to extract large amounts of proteins for biochemical assays (Chapter 15). It is also possible to create cell lines that continuously express a gene of interest, such as a fluorescently tagged or mutant version of a protein.

The major disadvantage to using immortalized cells is that these cells cannot be considered normal, in that they divide indefinitely and sometimes express unique gene patterns not found in any cell type *in vivo*. Therefore, they might not have the relevant attributes or functions of normal cells. Also, after several passages, cell characteristics can change and become even more different from those of a normal cell. Thus, it is important to periodically validate the characteristics of cultured cells and not use cells that have been passaged too many times.

Immortalized cell lines of neuronal origin can be used to study properties unique to neurons. Scientists have used neuronal cell lines to investigate processes that occur during differentiation in neurons, such as axon selection, guidance, and growth. However, most neuronal immortalized cell culture models are derived from tumors and are sometimes genomically abnormal. One popular neuronal cell line, called PC12, is a rat pheochromocytoma cell line derived from an adrenal gland tumor. The addition of nerve growth factor (NGF) causes PC12 cells to reversibly differentiate into a neuronal phenotype (Figure 14.2). These cells can synthesize dopamine, norepinephrine, and acetylcholine. PC12 cells have been used to study molecular phenomena associated with neuronal differentiation and have even been used

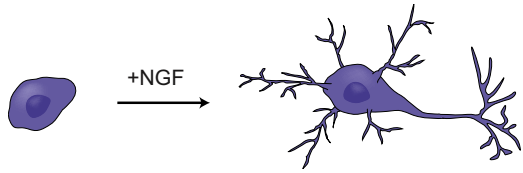

FIGURE 14.2 **Neuronal differentiation.** Addition of growth factor to PC12 cells causes them to differentiate into neuron-like cells that grow neurites and synthesize neurotransmitters.

in experiments to replace dopaminergic neurons in an animal model of Parkinson's disease. Neuroblastoma cell lines, like mouse Neuro2A, also express neurotransmitters and have been used in electrophysiology and neurodevelopment studies.

As useful as immortalized cell lines of neural origin can be for certain experiments, they show obviously abnormal traits, such as the unusual combination of neurotransmitters they produce (no normal neuron produces dopamine, norepinephrine, and acetylcholine in the same cell!). Therefore, it is advantageous, when possible, to use primary cultured cells—cells extracted from living animals.

PRIMARY CELL AND TISSUE CULTURE

Primary cell and tissue culture is derived from tissue removed directly from a living animal rather than immortalized cells that divide indefinitely (Figure 14.3). Primary tissue culture allows scientists to directly investigate cells of interest in a carefully controlled *in vitro* environment. These experiments can be especially

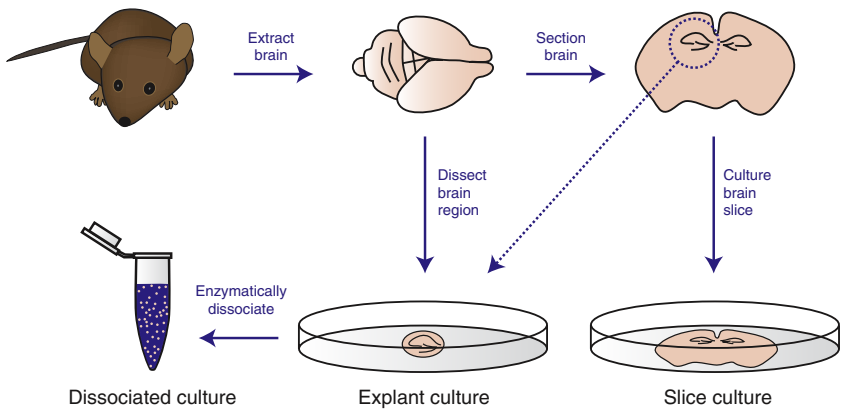

FIGURE 14.3 **Primary tissue cultures.** After a brain is removed from an animal, it can be cut into thin sections (usually using a vibratome) and kept alive in slice culture. Alternatively, either a brain slice or a brain region can be finely dissected to produce an explant culture. An explant culture can be enzymatically digested into single cells to produce a dissociated cell culture.

advantageous for a comparison between cells derived from a wild-type animal and cells derived from a genetically modified animal.

However, there are some disadvantages to using primary cultures (Table 14.2). Unlike immortalized cell lines, primary cultured cells have a limited lifetime. The age of the animal source influences the health and robustness of the cell culture: tissue from younger, embryonic or early postnatal animals survives better and tends to be healthier than tissue from older animals. Also, a population of primary cells will always be more heterogeneous than a culture of immortalized cells, no matter how careful the scientist was in extracting and purifying the cells of interest.

There are three main categories of primary tissue culture: slice cultures, explant cultures, and dissociated cultures.

Slice Cultures

Slice cultures maintain the structure and organization of the brain in a relatively thick (250–400 μm) section of brain tissue cut using a vibratome (Chapter 6). Slices provide greater access to and visibility of deep subcortical structures, like the hippocampus and thalamus, which are difficult to access *in vivo* (Figure 14.3). These slices can either be **acute cultures** that are used immediately (over a period of hours) or **organotypic slice cultures** that are maintained over multiple days. Acute slices are typically used for short-term electrophysiology experiments, while organotypic cultures are used to observe structural and morphological changes such as neuronal migration, axon outgrowth, or synapse formation.

Slices require an air/liquid interface to properly regulate gas exchange throughout the slice. Different protocols for culturing slices have varied success in preserving the structure and development of the tissue to parallel that seen *in vivo*.

TABLE 14.2 Immortalized Cell Lines versus Primary Cell Culture

	Advantages	Disadvantages
Immortalized cell lines	• Easier to use (grow, transfect, etc.) • Homogeneous • Fairly well characterized • Can create stable cell lines expressing gene of interest	• May not have the same properties as neurons or primary cell type of interest
Primary cell culture	• Relevant cell type, physiology, and circuitry	• Heterogeneous populations with high variability

Explant Cultures

Explant cultures consist of intact fragments of tissue (Figure 14.3). Although they do not necessarily preserve the precise organization and orientation of the endogenous nervous system, explants contain the same mixture of cell types. Unlike slices, which require an air interface for proper oxygenation, explant cultures can be submerged in the bath media. Explants are often used in co-culture assays and for studies on neurite outgrowth and neuronal migration.

Dissociated Cell Cultures

In **dissociated cultures**, neural tissue is separated into individual cells that are then grown on two-dimensional coated glass coverslips or within three-dimensional substrates (Figure 14.3). After extracting an animal's brain, specific regions can be micro-dissected to isolate a specific neuronal subtype (e.g., cortical, hippocampal). This region is then mechanically or enzymatically digested within a liquid suspension to separate individual cells from each other. The neurons are then removed from the suspension and plated onto a substrate on which the cells can attach and grow.

Neurons dissociated from different regions of the brain retain their initial identities. The morphological, molecular, and physiological properties of cell populations present in culture correspond closely to the characteristics of the cell population present in the region of origin in a living organism. With the proper growth factors and care, it is possible to maintain a dissociated culture for weeks, during which time cells acquire properties of mature neurons: they develop characteristic axons and dendrites, form synapses with one another, and express receptors and ion channels specific to their cell types, even producing spontaneous electrical activity. Dissociated neuronal cell cultures have been used to study neurite outgrowth, synapse formation, and electrophysiological parameters.

The ability to probe individual neurons, however, comes at the expense of losing the organization and connectivity critical to *in vivo* functions. Another limitation of dissociated cultures is the small quantity of cells relative to immortalized cell lines, which can make it difficult to perform biochemical experiments (Chapter 15) that require a high volume of starting material (e.g., millions of cells). Furthermore, most primary cell cultures are not homogeneous. Neuronal cultures are often mixtures of both glia and neurons that respond to different neurotransmitters, so identifying an individual population of cells can be difficult. To minimize heterogeneity, investigators usually attempt to dissect regions as precisely as possible to maximize the presence of the desired cell type in culture.

Various methods have been developed to purify specific cell populations. In a technique called **immunopanning**, a scientist coats the bottom of a plate with antibodies that recognize cell-surface markers on the outside of specific cell types. When heterogeneous populations of cells are added to the plate, the scientist can purify the cells of interest by allowing the cells to bind to the bottom

of the plate and then wash off the undesired, unbound cells. This technique has been used to culture oligodendrocyte precursors, retinal ganglion cells, and corticospinal motor neurons.

STEM CELL CULTURES

A **stem cell** is a **pluripotent** cell with the capacity to generate any cell type (e.g., neuron, muscle, blood) and an unlimited ability to renew itself. These cells are similar to immortalized cell lines in that they are theoretically able to propagate in culture continuously; however, they are also primary cells derived directly from a living organism.

Stem cells are classified by their source (e.g., embryonic stem (ES) cells, adult stem cells, induced pluripotent stem (iPS) cells), as well as the tissue they typically generate (e.g., neural stem cells (NSCs), hematopoietic stem cells, skin stem cells). Stem cells defined by the tissue they generate are **multipotent**—able to give rise to all types of cells found in the tissue and able to continuously self-renew. For example, NSCs can give rise to all three neural lineages—neurons, astrocytes, and oligodendrocytes—as well as additional NSCs. Stem cells are distinct from **progenitor cells**, which have a more limited capacity for self-renewal and may be **unipotent**, giving rise to a single cell type.

Stem cell culture is a form of dissociated primary culture. Therefore, stem cells must be cultured in the presence of growth factors (EGF and basic fibroblast growth factor (bFGF)) in specially formulated media that preserves their multipotency and ability to self-renew. Because scientists do not yet know all of the environmental components required to recreate *in vivo* conditions for preserving stem cell function, *in vitro* studies may not completely capture the effect of endogenous environmental influences.

Stem cell culture has a variety of uses in neuroscience. ES cells are examined for their ability to differentiate into specific neuronal subtypes. NSCs can be cultured to study the basic biology of development and aging. iPS cells can be used to generate clones of cells from patients with neurological disorders to characterize cellular and molecular changes in diseased neurons. While an extensive history and theory of stem cells already exists, basic stem cell biology is still a rapidly evolving field. Here we focus on specific applications of stem cell culture in neuroscience and describe common *in vitro* techniques used to identify stem cells.

Embryonic Stem Cells

Pluripotent **embryonic stem (ES) cells** can give rise to all tissues in an organism. Derived from the inner cell mass of a blastocyst embryo, culturing ES cells *in vitro* essentially traps the cells in a pluripotent state by growing them in the presence of factors that prevent the cells from differentiating. Investigators can reconstruct the environment of ES cells in a dish so that culture conditions

contain the specific molecules and mitogens that specify the formation of a neuron in the developing embryo.

Using special culture conditions, ES cells are first induced to become general **neural progenitors**, precursors that are committed to a neural fate. Once they have become neural progenitors, the specific molecules known to act during normal neuronal development are added to the culture to direct differentiation of specific neuron subtypes. For example, to make motor neurons, cells are exposed to high concentrations of sonic hedgehog (SHH) and retinoic acid, morphogens that pattern spinal cord motor neurons during development. To make midbrain dopaminergic neurons, cells are exposed to SHH and fibroblast growth factor 8 (FGF8), morphogens that specify dopaminergic fate during development.

Investigators monitor the progression of differentiation by examining the culture for activation of transcription factors relevant to development and the appearance of markers known to promote differentiation of specific neuronal subtypes. For example, when trying to induce midbrain dopaminergic neurons, scientists look for midbrain-specific transcription factors (Pitx3, En1, Lmx1b, Nurr1) and later for markers of mature dopamine neurons (TH, DAT, Girk2).

This process—from the initial establishment of the ES cells to their differentiation into specific neuronal subtypes—can take months. Also, despite attempts to create pure populations of a specific neuronal subtype, differentiated ES cell populations are still heterogeneous, with many other cells present in the culture. However, the ability to differentiate ES cells into specific neuronal cell types is useful for investigating therapies for a number of neurodegenerative disorders such as Parkinson's and Alzheimer's disease.

Neural Stem Cells

Multipotent **NSCs** exhibit self-renewal properties and the ability to differentiate into all neural subtypes. They can be extracted from regions of embryonic or adult brains, where they normally divide and give rise to neurons or glia. The embryonic brain contains many NSCs, while adult brains have far fewer NSCs. Furthermore, the ability of adult NSCs to produce neurons decreases with age.

Investigators typically culture adult NSCs from the subgranular zone of the dentate gyrus in the hippocampus and the subventricular zone in the wall of the lateral ventricles. After dissection, the region containing NSCs is dissociated and can be moderately purified through centrifugation. NSCs can also be grown and passaged in special media that promote NSC survival. By removing growth factors from the media, NSCs can be differentiated into neurons, astrocytes, and oligodendrocytes.

It is difficult to distinguish neural progenitors from NSCs, and scientists often confuse the terms. Both types of cells proliferate and express common sets of molecular markers (e.g., nestin, Pax6). Therefore, functional assays must also be used to determine the identity of cultured cells. Two defining features of

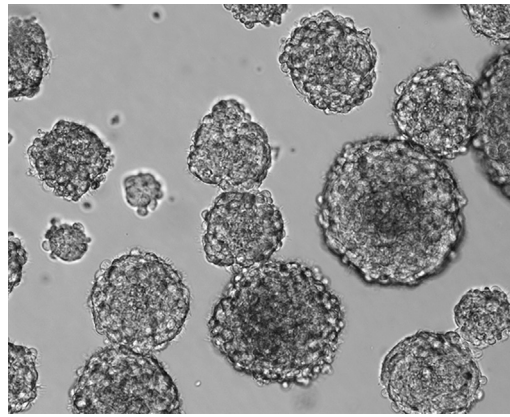

FIGURE 14.4 Neurospheres. Dividing neural progenitor and stem cells form spheres in culture. *Courtesy of Dr Victoria Rafalski.*

NSC cultures that can be tested to confirm cellular identity are (1) the ability to give rise to all three types of the neural lineage (multipotency) and (2) the ability to propagate more NSCs (self-renewal).

Self-renewal is often examined by performing **primary neurosphere** and **secondary neurosphere assays**. When NSCs are cultured in nonadherent conditions in the presence of the growth factors EGF and bFGF, they give rise to **neurospheres**, balls of dividing cells (Figure 14.4). However, neurospheres form as a general result of dividing neural precursors, including progenitor cells that do not continuously self-renew. Therefore, while this assay is frequently used, it does not definitively distinguish between stem and progenitor cells. The secondary neurosphere assay involves culturing cells from established neurospheres to see if the cells generated by the first neurosphere are able to continue proliferating, an indicator of self-renewal. These assays can be difficult to quantify, as cells and spheres can each fuse, so a sphere may not necessarily form from a single NSC. Also, neurospheres are species-specific; rat and human NSCs do not form neurospheres as often as mouse NSCs.

Induced Pluripotent Stem Cells

iPS cells are differentiated cells that scientists have reverted back to a stem cell state. A variety of strategies have been employed to carry out this feat. One common strategy is to use viruses to introduce specific transcription factors into differentiated somatic cells, usually fibroblasts. The presence of these transcription factors seems to allow these cells to exhibit stem cell behaviors. Other strategies employ a combination of viral delivery and chemical manipulations to coax somatic cells into a pluripotent state.

Once iPS cells are generated, they are examined for characteristics of "stemness" by comparing them to ES cells in various ways. Scientists

typically examine transcription factor profiles, measuring the presence of markers such as Oct4, Nanog, Sox2, AP, SSEA4, and TRA-1-80 using immunohistochemistry (Chapter 6) and reverse transcription polymerase chain reaction techniques (Chapter 10). The potential for these iPS cells to differentiate is measured by driving cells into specific lineages using established protocols. The gold standard of determining whether iPS cells are like stem cells is determining whether these cells retain the ability to generate chimeric mice (Chapter 13).

In neuroscience, the ability to reprogram fully differentiated cells into an ES-like state opens up the possibility of modeling human disease *in vitro*, directly from patient-derived cells. These cells could reveal the cellular and molecular pathogenesis of diseases such as Parkinson's and autism spectrum disorders. iPS cells may also be used for therapeutic purposes: for example, generating new neural cell types for transplantation from a patient's own somatic cells. Work has also progressed in bypassing the iPS cell stage to directly reprogram somatic cells into functional neurons.

MANIPULATING CELLS IN CULTURE

One of the major advantages of studying the nervous system *in vitro* is the ability to control the cellular environment. In addition to other methods of neuromodulation (Chapter 8), scientists can manipulate neurons in culture using several methods.

Transfection and Infection

Transfection and **infection** are the processes of delivering DNA to cells using nonviral and viral methods, respectively. One of the major benefits of cell culture systems, especially dissociated cultures, is that they are very amenable to both DNA delivery methods. Therefore, it is usually easy to introduce recombinant DNA molecules into cultured neurons to change gene/protein expression and alter cellular physiology. Transfection and infection procedures are discussed in much greater detail in Chapter 11.

Co-Culture Systems

Co-culturing allows a variety of cell types to be cultured together to examine the effect of one culture system on another (Figure 14.5). This procedure is useful when examining the effect of one type of tissue on another, one region of the brain on another, or how a particular secreted molecule leads to changes in neural development or physiology. For example, co-cultures of different regions of spinal cord explants initially revealed differing effects on the ability to attract or repel neurite outgrowth. Biochemical purification from explants in co-culture experiments led to the identification of specific molecules that could then be introduced

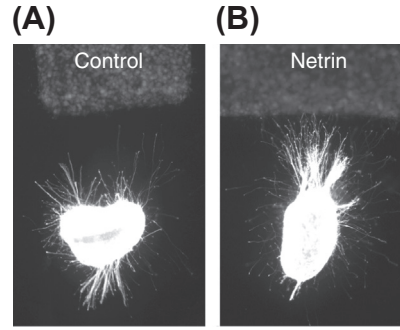

FIGURE 14.5 The use of co-cultures to study guidance cues. In this example, midbrain explants are placed in culture medium. COS cells (top) transfected with a control plasmid (A) do not influence the direction of axon outgrowth from the explants. However, COS cells transfected with netrin-1 (B) sufficiently attract axons. *Courtesy of Dr Jie Li and Dr Mary Hynes.*

into immortalized cell lines to express and secrete these molecular guidance cues. Transfected cell lines could then be co-cultured with spinal cord explant cultures to examine the neurite outgrowth response of the spinal cord neurons to specific guidance cues. Such approaches have deciphered a large variety of chemoattractants and chemorepellants that have since been validated *in vivo*.

Pharmacology

Because of the unparalleled access to the medium in cell culture conditions, it is relatively easy to add pharmacological agents to culture media to affect proteins, ion channels, and receptors of interest. See Chapter 8 for a detailed description of pharmacological techniques.

Antibody Interference

Antibodies are molecules that bind with high affinity to antigens, usually parts of a protein. In nature, these antibodies function as part of the immune system. In the laboratory, antibodies can be used in many different assays to study protein expression and binding partners (Chapter 15). If the antigen of an antibody is accessible in living cells, it is also sometimes possible to block protein function by applying antibodies against the protein of interest. These antibodies bind the protein at a site critical for its normal function, thus inhibiting function *in vitro* or *in vivo*.

CONCLUSION

The ability to grow cells and tissues outside of a living organism allows scientists much greater access and control over the cellular environment compared with *in vivo* conditions. Therefore, scientists can investigate the cellular and molecular mechanisms of nervous system function with great detail. The tools and techniques of *in vitro* culture techniques can provide many informative results, but ultimately this knowledge must be tested in the intact nervous system to fully understand how a biological process works in a living organism.

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