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Stem Cells and Organ Transplantation: Resetting Our Biological Clocks

Backward, turn backward, O Time in your flight...

Elizabeth Akers Allen
“Rock Me to Sleep” (1860)

The human body has only a limited ability to repair itself. Illness, injury, and aging can overwhelm its built-in capability to replace dysfunctional, damaged, or destroyed tissues. We can at best only partly regenerate our organs and cannot grow back a whole limb.

This chapter focuses on two major approaches to renewing our bodies. In science fiction, futuristic medicine developed by humans or extraterrestrials might be able to regrow or replace body parts far beyond current abilities. One avenue of research that shows great promise for translating those imagined capabilities into actual practice involves the use of “stem cells.” They are a particular class of cells found in the human body before and after birth.

There are many types of stem cells, each with its own particular capabilities. All share the common trait of being able to transform into one or more other types of cells, including the specialized, “differentiated” ones present in various tissues and organs (e.g. neurons in the brain). Stem cells could potentially be directed to change into different varieties needed to replace a person’s own malfunctioning or destroyed cells.

The extensive role stem cells play in the human body’s development and health, and particularly their potential therapeutic uses, have been recognized only relatively recently. Thus, while science fiction works have long employed the concept of regrowing body parts, their use of stem cells as the means for doing that is also more recent, paralleling growth of real-life knowledge about them. Stem cell research is a very active field, with major discoveries made over the past few decades and others likely to come in the near future. It is an area that shows great promise for eventually changing some science fiction medicine into everyday clinical practice.

A second method that is already used to replace some body parts is tissue and organ transplantation. This is a much more established technique that ba-

sically involves “swapping out” a damaged body part for a healthy one. Typically this involves using tissue or an organ obtained from a deceased donor. However, in some cases (e.g. bone marrow and kidney transplants) the donor may be alive. Occasionally a person’s own tissue (such as bone marrow) may be obtained for later transplantation into his or her own body.

In science fiction organ transplantation be used for benign or sinister purposes. For example, standard medical practice requires that the latter meet strict criteria for brain death before donating an organ essential to life, such as a heart. It also requires that a person previously gave consent to become an organ donor or that permission to donate organs be given by an appropriate individual such as a spouse or family member.

However, both in medical thrillers and science fiction the ethical principles used for such “harvesting” may be considerably more lax. Robin Cook’s 1977 novel *Coma* involves patients being deliberately rendered brain dead and used as spare parts for transplants. Larry Niven’s “Known Space” works such as “The Organleggers” (1969, later republished as “Death by Ecstasy”) depict wholesale “organlegging” and other methods such as extensive use of the death penalty for increasingly less violent crimes such as income tax evasion to keep a larger supply of organs available.

Both stem cells and transplantation techniques offer potential ways to improve health and increase life expectancy. As we will see, these two methods may be used together, such as by transplanting tissue (and, perhaps, someday whole organs) created using stem cells into a person. However, each one has its own particular uses and challenges.

13.1 Types of Stem Cells

Our bodies contain many “mature” cells that normally do not demonstrate the ability to change into or produce other types of cells. Once red blood cells, neurons, myocytes (heart muscle cells, also known as cardiomyocytes), and many other varieties develop they remain as those same types of cells for the rest of their existence. Cells that have reached the end-stage of their development and are no longer able to divide are called “terminally differentiated.”^[1] For example, a red blood cell ultimately loses its nucleus and thus its ability to reproduce. Mature neurons and myocytes, while retaining their nuclei, are also thought to not divide in adults. Myocytes do, however, increase significantly in size from birth to adulthood, thus increasing the overall dimensions of the heart.

Stem cells are at the opposite end of the spectrum, with far greater abilities to reproduce and transform into other types of cells [2–4]. The human body

uses them to develop, grow, and repair itself from the very beginning of life until its end. There are many varieties of stem cells, each with its own origin, location within the body, and range of capabilities. However, all of them share certain characteristics. Stem cells can divide and proliferate throughout most if not all of a person's entire lifetime. They can differentiate into some or all of the kinds of cells that create the various tissues in our bodies.

Stem cells are also self-renewing. They can replenish their numbers via either "symmetric" or "asymmetric" cell division [5, 6]. In symmetric cell division the stem cell can divide into either two "daughter" cells identical to the original, or into two cells that then differentiate into another kind of cell. In the former case the total number of stem cells increases, while in the latter the body gains new cells for a particular tissue. Asymmetric division produces one daughter stem cell and a second cell that then differentiates into another variety. This maintains the same total number of stem cells while also increasing that of a different type.

Embryonic stem cells (ESCs) are the class of stem cells with the greatest inherent ability to divide and form the widest range of tissues [7]. As the name implies they are present in a human (or non-human animal's) embryo, the stage of development in our species extending from fertilization (the union of a sperm cell and ovum to produce an initially single-celled "zygote") to about 8 weeks post-fertilization. In humans, the zygote and the cells created by its first two divisions are "totipotent," or "omnipotent" [7–9]. This means they have the ability to differentiate into any of the different classes of cells that the human body can have, as well as the tissue that will form the embryo's attachment to the mother's uterus after implantation. The "potent" part of these and subsequent terms refer to a cell's ability to change into another type.

An embryo between about 5–9 days after fertilization is called a "blastocyst." It consists of up to several hundred cells that have differentiated into two basic classes of cells. One class includes the cells that will ultimately help form the "extra-embryonic" tissues of the placenta. These "trophoblasts" form the outer layer of the blastocyst. The ESCs are located below that layer. The latter are "pluripotent" [10]. They can differentiate into any human cell except the ones that help make the placenta.

More technically, ESCs can produce cells arising from any of the three "germ" layers in our bodies—the endoderm, mesoderm, and ectoderm. The endoderm gives rise to the various kinds of cells that line nearly all of the gastrointestinal tract; the trachea, bronchi, and alveoli of the lungs; the urinary bladder; and parts of the urethra and thyroid gland. The mesoderm produces connective tissues such as cartilage and bone, all types of muscle cells, red and white blood cells, adipose (fat) cells, and germ cells (sperm and oocytes). The ectoderm differentiates into the cells of the brain and other parts of the

nervous system, as well as into skin, hair, sweat glands, and the lining of the mouth.

ESCs can be obtained by extracting them from a blastocyst [11] A method for obtaining these cells from human embryos was first described in 1998 [12]. The standard technique for doing this results in the destruction of the embryo, raising ethical questions regarding “harvesting” such cells from human embryos. ESCs obtained in this way and maintained in cultures have the ability to divide indefinitely and develop into any type of human cell. This gives them the greatest potential flexibility for generating tissues or perhaps even entire organs that could be used to replace a person’s damaged ones. However, as will be discussed shortly, ESCs have other characteristics that limit such use.

Fetal stem cells are present in the organs of a fetus, the stage of development between 8 weeks following fertilization to birth. These stem cells are “multipotent.” They are limited to developing into closely related classes of cells within a single germ line. For example, those found in the liver of a fetus can develop into more than one kind of cell, but only those types found in the liver and not elsewhere in the body.

Following birth, our bodies retain reserves of “adult stem cells” in most and perhaps all tissues [6, 8, 13, 14] At least some of these too are thought to be multipotent. For example, neural stem cells can differentiate into neurons as well as various supporting cells such as oligodendrocytes and astrocytes. They are present in specific areas of the brain and also found within the spinal cord [15].

Mesenchymal stem cells are derived from the mesoderm germ line. They are located in multiple tissues in our bodies throughout life, including bone marrow, bone, adipose tissue, and blood [6, 16, 18] They are thought to assist in replacing mature, differentiated cells there and elsewhere by turning into the types needed within their specific range of transformation. Mesenchymal stem cells can differentiate into osteocytes (bone cells), chondrocytes (cells producing cartilage), fibroblasts, adipocytes (fat cells), myocytes, and hepatic (liver) cells [16, 17] Recent reports also suggest that, under certain conditions, mesenchymal stem cells can even be induced to “transdifferentiate” into cells of a different germ line such as neural tissue, which derives from the ectoderm germ line [18]

Mesenchymal stem cells are also present in umbilical cord blood and especially in the cord tissue itself. By saving and preserving cord blood and tissue, these and other types of stem cells can be available for further study should the person develop a disease or need them for possible future tissue regeneration [13].

Other types of adult stem cells are “oligopotent.” They produce two or more types of cells within a single, specific tissue. For example, hematopoietic stem cells were first identified over 50 years ago [5]. They are capable of differentiating into all types of myeloid (e.g. red blood cells and most kinds of white blood cells) and lymphoid (e.g. lymphocytes) cell lines. Hematopoietic stem cells are concentrated in blood obtained from the umbilical cord at birth and also reside in bone marrow throughout life [19]. Like mesenchymal stem cells, if they are preserved in stored umbilical blood they can potentially be used later in a person’s life for study and therapeutic use should that individual develop a particular disease (e.g. one of certain kinds of leukemia) [20].

Hematopoietic stem cells constantly produce new blood cells to replace those continuously lost through “old age” or destruction. Red blood cells have an average lifespan of about 120 days, while most types of white blood cells have lifetimes ranging from a few days to weeks. Without hematopoietic stem cells to replenish their numbers our bodies would quickly “run out” of these essential blood cells.

Finally, some adult stem cells are “unipotent.” They produce only a single type of mature cell and typically reside only in the tissue containing that variety of cell. Thus, muscle stem cells are present in skeletal muscles and can turn into mature muscle cells (myocytes) if needed. Some unipotent and oligopotent cells are called “progenitor cells.” They may be quiescent—neither dividing nor differentiating—unless called upon to do that by injury to the tissue where they reside. These progenitor cells can represent an intermediate stage between stem cells and fully differentiated ones. Unlike stem cells they cannot usually self-renew, and they typically differentiate into more mature cells soon after they are produced [2].

The role of adult stem cells varies depending on their types and locations. As mentioned previously, hematopoietic stem cells are continuously replicating and differentiating due to the high turnover rate of blood cells. Stem cells in the lungs, liver, hair follicles, and gastrointestinal tract also do this to replace cells within them having short life cycles [5]

On the other hand, stem cells in organs and tissues such as the heart, skeletal muscles, pancreas, and nervous system are predominantly dormant. They form new, mature cells of only a specific type, and at a very slow pace that may increase due to an acute need such as damage to those mature cells. In some cases their rate of turnover is so slow that, in the case of the heart and nervous tissue, it was thought until recently that those organs had no ability to create new mature cells following birth. This is in contrast to early (e.g. embryonic) stages when stem cells divide and proliferate rapidly to ultimately produce all the differentiated cells that develop during the active growth process [21].

In short, small reserves of different types of adult stem cells are present in at least most tissues throughout life. They can help replace damaged, destroyed, or diseased cells with widely varying degrees of efficiency [22]. However, with aging the number of these stem cells and how well they function can decline [21, 23]. The latter may be associated with damage to their DNA, environmental changes involving the cells, alterations in their physiology, and reduction in their ability to suppress tumor formation [15, 21].

For example, as new “daughter” stem cells are produced they may not always be exact copies of their “parent” due to mutations. Some may also eventually lose the ability to proliferate due to shortening of telomeres (described in Chap. 9) and other causes [21]. After a certain point they can become senescent—still alive but with reduced function and no longer dividing. If they become sufficiently “defective” over time they may undergo “apoptosis,” a process in which a cell deliberately self-destructs, thus decreasing the total number of stem cells if they are not replaced. And as with other cells, one or more mutations could also potentially make them turn cancerous (more about this soon.) This may be more likely to occur after the stem cell has undergone many divisions, perhaps due to each division having a certain risk of malignant transformation and the “odds” eventually catching up with the cell, or by simply accumulating enough deleterious mutations over time to ultimately turn them into a cancer.

Another way stem cells might deal with defects produced by their aging or when they are subjected to stress is by “autophagy.” This means that the cells can “digest” their own defective organelles and other components, recycling them and synthesizing new ones. However, with further aging stem cells may lose the ability to prolong their “healthy” lives via autophagy, leading to them being destroyed or becoming senescent without being replaced by normal offspring or, once again, accumulating enough defects to make them turn malignant [21, 22].

A reduced pool of effective stem cells could partly explain the body’s diminished ability to replace damaged mature cells in various tissues with aging. Even at best the number of stem cells in various parts of the body is small compared to other types. Also, while their life expectancy is typically much longer than other types of cells, it is still finite. Thus, if stem cells can in fact divide only a certain number of times over a person’s life before they become senescent, die, or become dysfunctional (including turning cancerous), their ability to repair or regenerate tissue will also be limited [5].

Adult stem cells that are normally dormant occupy their own “microenvironment” within tissues. Chemical and other changes within that microenvironment act as stimuli for them to “spring into action” and respond to tissue injury by initiating various repair mechanisms. An important area of current

research is to determine what factors, either intrinsic to the cells themselves or associated with their microenvironment, make some types of adult stem cells (e.g. hematopoietic stem cells) so efficient at replacing mature cells and regenerating the corresponding tissues while others (e.g. neural and cardiac stem cells) show considerably more limited capabilities [5].

For example, when I went to medical school in the mid-1970s it was thought that adults had a finite number of myocytes that, if lost by damage or disease, were not replaced. However, it was first reported in 2003 that cardiac stem cells are indeed present in the heart, although in limited numbers [24, 25]. It is now thought that they actually replace about 1 % of myocytes per year, and that about 40 % of those latter cells in an adult's heart were created after birth [26]. Put another way, it has been estimated that about half of the heart's mass has been renewed by age 50. However, the rate at which this "turnover" occurs is thought to decrease with age [13].

Why cardiac stem cells can perform this "routine maintenance" but do not seem to "pick up the pace" and perform a significant amount of repair work when the heart suffers major destruction of myocytes (e.g. at the time of a myocardial infarction) is unknown. Finding some way to stimulate them to act more rapidly and extensively to repair large areas of a damaged heart would be highly desirable, but whether this is possible and, if so, how to do it have yet to be determined.

Some adult stem cells can be harvested relatively easily from a person's body. For example, hematopoietic stem cells can be obtained from a person's bone marrow, where they make up about 2–4 % of the cells there [27]. After administering a local anesthetic to the skin and surrounding tissue, a needle is inserted into a bone containing accessible marrow, usually the sternum (breastbone) or a part of the pelvic bone. A small amount of fluid marrow is then drawn up into a syringe. A more solid sample of marrow cells can be obtained from the pelvic bone as a biopsy using a hollow needle that goes deeper into the bone. In either case the blood cells obtained will be at various stages of development, and a small percentage will be stem cells.

Hematopoietic stem cells obtained from bone marrow and other sources are, in fact, currently used to treat a number of leukemias, lymphomas (a kind of cancer derived from lymphocytes, a type of white blood cell), other blood disorders, and some other conditions. One technique is to first partially or completely destroy a person's own "unhealthy" blood cell-producing system (including the immune system) via chemotherapy and radiation at doses that do not significantly injure other body tissues. The harvested normal bone marrow or stem cells can then be infused intravenously, where they migrate to the usual sites of bone marrow formation and establish themselves there, proliferating and restoring the person's hematopoietic and immune system.

The bone marrow and stem cells used for transplantation can be obtained from either the person being treated or from another individual who is a close match regarding shared antigens, particularly those of the “human leukocyte antigen” (HLA) system. When the transplant source is “autologous” (derived from the patient) the cells have a significantly lower risk of producing an immune response than when they are “allogenic” (derived from a donor). As described in Chap. 12, the latter can be associated with a “graft-versus-host” reaction in which the transplanted cells recognize the new host’s cells as foreign and produce an immune response against them. Autologous transplants also tend to restore blood cell production and immune system function more quickly than allogenic ones. However, autologous transplants may be associated with a greater risk for recurrence of the patient’s original disease [19].

Hematopoietic stem cells can also be derived from other sources. They are present in circulating blood, where they can be extracted via filtering techniques. As mentioned previously they can be obtained from umbilical cord blood, and even from amniotic fluid obtained at birth. In the latter cases, the blood and/or amniotic fluid must be collected at birth and cryogenically preserved (see Chap. 7) for long-term storage.

While hematopoietic stem cells can only differentiate into blood cells, mesenchymal stem cells are potentially more versatile due to their ability to generate a wider variety of useful tissues [28]. The particular types of cells they can differentiate into depend on the cellular environment into which they are introduced. Mesenchymal stem cells also have a natural ability to migrate to an area of injury within the body, attracted by chemicals such as cytokines released by damaged cells. After reaching their destination they can promote healing by helping to suppress inflammation and differentiating into cells that replace damaged or destroyed ones [16, 29].

Like hematopoietic stem cells, mesenchymal stem cells can be obtained from bone marrow.¹ Unfortunately they are present there in only very small numbers, comprising somewhere between 0.001–0.1 % of bone marrow cells [17, 27]. Once they are obtained and their numbers possibly augmented by culturing them, they can be used to treat a patient by giving them back via an intravenous or arterial infusion, or by a direct injection into the target tissue. However, the best ways to administer them for a particular application are still being assessed [29].

A point in favor of mesenchymal stem cells is that they have a relatively low tendency to provoke an immune response when transplanted from one person to another. This raises the possibility of storing them in “cell banks” where

¹ For completeness sake it should be noted that these cells are also sometimes referred to as “mesenchymal stromal cells.”

their numbers can be increased via culture techniques for clinical use when needed [24]. Nonetheless, under certain circumstances they can increase their likelihood of being recognized as “foreign,” so the actual feasibility of this approach is still uncertain [29].

So far more than 200 clinical trials involving mesenchymal stem cells have been conducted in the United States. However, the total number of patients studied is only a little over 2000, and these trials varied widely regarding the procedures used and the diseases being treated. How well mesenchymal stem cells can be used to help repair or replace tissues is a dynamic but still uncertain area of research, with overall results that are said to be currently both inconsistent and inconclusive [17].

Due to the type of tissues they can form, including bone, cartilage, and fat, mesenchymal stem cells may have particular applicability to medical fields such as orthopedics and plastic surgery [30]. For example, those derived from adipose tissue might be transplanted to stimulate creation of fat in areas of the face and limbs where it has been destroyed or is deficient, thus providing cosmetic and functional benefit. They might also be used for bone regeneration, producing new cartilage for damaged joints, assisting wound healing, rejuvenating skin, and perhaps even helping injured peripheral nerves to regenerate [30].

A practical issue for using adult stem cells or, for that matter, any other type is how many of them need to be introduced into a target tissue. Use too few and the treatment might be ineffective, while too high a dose might increase the odds of the cells having undesirable effects such as interfering with the function of normal cells or provoking an immune response.

A commonly used dose for mesenchymal stem cells is about $1\text{--}2 \times 10^6$ cells for each kilogram of body weight. However, as described previously the number of mesenchymal stem cells in bone marrow is very low, and obtaining enough to give that dose typically requires obtaining a small sample and culturing it. While these cells can replicate quickly, increasing their number a thousandfold within several weeks, there is the risk of them being damaged during that time or exhausting their extensive but finite ability to divide. Protocols for optimally processing them for therapeutic uses are still being studied [17].

Some areas, such as the brain, may not only require a large number of stem cells or differentiated cells derived from them for purposes of repair or rejuvenation but would also face other challenges. For example, the microenvironment in parts of the brain requiring treatment may not be hospitable for those types of cells or for initiating production of new neurons or supporting cells. Likewise, the newly created cells would also have to integrate into the neurons and other cells already there in a functionally meaningful way, e.g. forming

synaptic connections. And if the conditions that produced the original injury or disease were still present, those “young,” healthy cells may also be vulnerable to them too [15].

It is also important to determine at what point in a particular disease process introducing stem cells would do the most good. For example, studies involving infusions of mesenchymal stem cells after a myocardial infarction showed better improvement in heart function when the cells were administered 5 or more days after the injury rather than earlier [24, 31]. This might have been due to injured areas of the heart having a more hostile environment early on due to release of harmful substances, insufficient oxygen supply, etc. associated with the infarction.

Stem cells administered to a damaged tissue might help repair it by creating new, healthy cells that are then incorporated into the tissue. However, in some cases the predominant way they can help repair it is by their mere presence altering the microenvironment and stimulating the tissue to repair itself by various means, so-called “paracrine” effects [18, 25, 32]. This can include secreting substances that help promote healing [16].

For example, although stem cells derived from bone marrow have been introduced into damaged hearts either through infusion via a coronary artery or direct injection, the reported resulting improvements in heart function have ranged from none to at best mild [24, 27]. Even when improvement was found it might be only transient. In these particular instances any benefits may indeed have been due to temporary improvements caused by the presence of these stem cells rather than generation of a significant number of new, healthy myocytes.

An alternative method first used in 2001 involved transplanting many hundreds of millions of myoblasts—undifferentiated cells derived from a person’s skeletal muscle—into that same individual’s heart [24]. While a detectable improvement in heart function was sometimes reported as occurring, these cells had the decidedly undesirable effect in some patients of producing serious ventricular arrhythmias (abnormal heart rhythms). This was thought to be due to the skeletal muscle cells not integrating with the “native” myocytes from an electrical standpoint and thus producing areas where those arrhythmias could start. This issue further demonstrates the all too common problem of a new treatment having unforeseen harmful effects.

Initial studies in small numbers of patients using actual cardiac stem cells and similar heart-derived cells have been somewhat more promising [26]. However, this area of research will require much more study before definitive results can be obtained.

Other types of stem cells, such as those derived from adipose tissue, also represent potential venues for research [32]. In particular neural stem cells and

other types of pluripotent cells are being studied as possible means to treat a wide variety of common neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, as well as other neurological problems (e.g. spinal cord injuries) [33–35]. Specifically, it is hoped that neural stem cells could generate new neurons, myelin-producing cells, or the cells of the eye's retina based on a person's needs. Fetal and adult neural stem cells could also potentially be used for these purposes. An important issue regarding harvesting adult neural stem cells from a person is that those cells are located in discrete parts of the brain, which are obviously considerably less easy to safely access than other types of adult stem cells (e.g. the hematopoietic and mesenchymal varieties obtained from bone marrow) [36].

Another possible source for neural stem cells or more differentiated (e.g. progenitor) neurological cells is to create them from ESCs or mesenchymal stem cells. Fortunately, like their mesenchymal counterparts, neural stem cells have the ability to migrate to an area of injury or other site where they are needed. Thus, if an adequate number of them were introduced into the body, they would potentially “know” where to go to do the most good. However, also like mesenchymal stem cells, any neural stem or other cells obtained will likely need to be cultured to increase their numbers to a level high enough to potentially produce a beneficial clinical effect. Here again this poses the risks of cell death, the stem cells differentiating prematurely or into the wrong kind of cell, etc. Developing better culture methods to reduce such issues is a critical preliminary step in transplanting these cells for therapeutic uses.

13.2 Reprogramming Cells

As described in Chap. 10, experiments in the 1950s provided the first steps in demonstrating that somatic cells could be “reprogrammed” to an earlier stage of development [37, 38]. For example, a study published in 1958 reported the creation of cloned tadpoles after nuclei derived from the intestinal cells of a frog were introduced into enucleated egg cells [38]. This was an important milestone in demonstrating that “mature” cells could be reprogrammed back to an earlier state of development. The creation of Dolly the sheep and subsequent mammalian clones was a further development of that somatic cell nuclear transfer method [7, 9, 39].

Induced pluripotent stem cells (iPSCs) are differentiated cells from an adult animal (e.g. a mouse or human) that, using one of a variety of methods, change into ones with properties similar to pluripotent ESCs [3, 7, 9, 40, 42]. This was first done in 2006 using fibroblasts derived from mice [43]. The fibroblasts had four genes encoding for “transcription factors” with the

unpoetic names *Oct3/4*, *Sox2*, *Klf4*, and *c-Myc* introduced into them using a genetic engineering technique involving a retrovirus.

These four genes reprogrammed the fibroblasts so that they could then be induced to form cells from any of the three germ lines, just as ESCs can. A follow-up study from those same investigators published in 2007 used that technique on human fibroblasts and produced similar findings [44].² Subsequent work has shown that other transcription factors, with equally non-intuitive designations such as *Nanog*, can also be involved in maintaining pluripotency in ESCs and other cells [45, 46]. Other somatic cells besides fibroblasts, such as keratinocytes, neural cells, and lymphocytes have also been used to generate iPSCs [47].

The exact details of what changes occur at the molecular level when somatic cells are reprogrammed into iPSCs are not completely established. Basic research studies are, however, continuing to identify what happens during this process [48]. A greater understanding of the underlying mechanisms involved could lead to more efficient production of iPSCs as well as identifying what types of somatic cells might be most amenable to this process.

Recent studies indicate that iPSCs and ESCs are not entirely identical from either a genetic or epigenetic standpoint, but what those differences mean functionally is still being investigated [49–53]. One issue with producing iPSCs is that reprogramming is not an all-or-nothing process but involves a series of changes. The techniques used to create an iPSC may leave the original somatic cell (e.g. a fibroblast) only partially reprogrammed and never reaching the stage where it would be able to differentiate into the desired new cell type [7]. Even somatic cells that do become pluripotent may retain some of their original characteristics as well as have changes induced by the reprogramming process itself, with uncertain effects on the functional status and other attributes of differentiated cells produced by them [7].

One method used to demonstrate that iPSCs are truly pluripotent is to use “tetraploid complementation” in animal models (e.g. mice) [54, 55]. In this technique two embryos, each at an extremely early stage of development when it consists of only two cells, are fused together. The resulting single cell is “tetraploid”—that is, it has two pairs of each type of “somatic” chromosome (those that are not X or Y chromosomes) instead of the single pair in a normal “diploid” cell. When either ESCs or iPSCs are combined with the tetraploid cell at a stage no later than a blastocyst, the tetraploid components form the

² The 2012 Nobel Prize in Physiology or Medicine was awarded jointly to the lead investigator for these studies, Dr. Shinya Yamanaka, and to Sir John B. Gurdon, who pioneered the previously mentioned somatic cell nuclear transfer technique in frogs.

extra-embryonic tissues (e.g. the placenta) while the diploid ESC or iPSC provides the genetic material to create the animal.

Such “combined” cells have been implanted into mice and produced viable offspring. The latter is an approximate clone of the animal from which the ESC or iPSC was initially derived. However, production of the iPSCs produces enough genetic and epigenetic changes in the “donor” cells that the offspring will not be identical to the “parent.” Nonetheless, this method represents a possible alternative to somatic cell nuclear transfer techniques for producing “clones.”

It should be noted, however, that the efficiency of using this technique to create offspring surviving to birth has been reported to be low (0.3–13%) [55] and that many of those created did not survive long after birth. Thus, any application of such techniques to humans would raise at least the same ethical issues as somatic cell nuclear transfer [56].

iPSCs are now being used for research purposes to create a “disease-in-a-dish” [13, 16, 25, 39, 52, 57–59]. For example, fibroblasts can be obtained from a person by removing a small sample of skin and culturing those cells. They can then be changed into iPSCs that in turn are induced to form cells of a tissue affected by a genetic or other disorder. Those cells can then be studied to determine what functional or physiological issues are associated with the disease. For example, myocardiocytes derived from iPSCs originating in patients with diseases that can produce life-threatening arrhythmias have been studied to see what changes in normal electrochemical processes or other abnormalities they have. A more direct way to make such assessments is to compare iPSCs produced from a person with a genetic disorder to “normal” control ones [57].

iPSCs have also been derived from cancer cells, including those from various types of leukemias and gastrointestinal cancers, to better understand their individual characteristics [40]. Interestingly, when cancer cells are made pluripotent they may lose at least some of their ability to produce tumors, and differentiated cells derived from them may also lack this and other hallmarks of cancer [39]. It is hoped that the knowledge obtained from these methods can be used to devise better treatment strategies, such as developing new drugs designed to correct or at least ameliorate the underlying defect(s) present in abnormal tissue or to destroy cancer cells.

ESCs can also be used to differentiate into particular types of cells used for disease modeling and drug testing [16]. However, iPSCs have the advantage of being “personalized” for a particular patient—derived from that individual’s own cells—rather than providing the more general information ESCs give [40]. Thus, iPSCs might help identify very specific issues with genetic

composition and cell function in an individual and thus help better direct potential therapies.

iPSCs could potentially be combined with gene therapy to treat some medical problems. One way of doing this could be to take cells such as skin fibroblasts and convert them outside the body (“in vitro”) to iPSCs. The original genetic defect would then be corrected by gene therapy and the iPSCs then induced while still in culture to differentiate into the required type of cell—e.g. a blood or muscle cell, or one that is part of the nervous system [60]. This might be done by producing appropriate chemical and other conditions in the culture where the iPSCs are growing [27]. The “processed” cells could then be injected back in the person’s body where they hopefully would then function properly. Alternatively the “repaired” iPSCs could be injected into the person at a location conducive to them differentiating into the “right” type of cells. Perhaps ideally some of these iPSCs would not initially differentiate but remain as an ongoing “pool” to potentially replace any mature tissue cells that are lost in the future [1].

Another potential approach might be to reprogram a fibroblast only partially so that, instead of becoming pluripotent and thus an iPSC, it travels only far enough back along its developmental path so that it can be changed directly into a myocyte or other type of differentiated cell [27, 50]. This can, in fact, be accomplished by a process mentioned previously, “transdifferentiation,” in which a fibroblast could be turned into a myoblast, a pancreatic cell into a hepatocyte, etc [50]. Because fibroblasts are present in the scar tissue that forms in the heart after a myocardial infarction, one possible application of this technique would be to reprogram the fibroblasts within the scar itself to turn into myocytes. Whether this can actually be successfully accomplished remains to be determined, however.

One potential major advantage of using iPSCs as a source of replacement tissue is that, because they originate in a person’s own body, they would not be expected to produce an immune response as would any type of material deemed by the body to be “foreign,” such as ESCs. However, the new gene introduced into modified iPSCs or differentiated tissue derived from them might itself be considered foreign, and the possibility that it could therefore elicit an immune response must still be taken into account.

Moreover, as with any type of gene therapy a number of these cells large enough to be effective would need to be created; the method used to introduce a new gene must be sufficiently efficient to produce an adequate number of corrected cells; and the vector used to introduce that gene should not cause changes to the DNA of the cell that might cause delayed adverse effects, e.g. cancer. Once these cells are introduced into the body they must also do their intended “jobs” well enough to clinically benefit the patient. This might be an

easier task for blood cells, which can be present in multiple areas of the body and do not need to organize into or interact in specific ways with “solid” tissues, such as nerves or the brain’s synaptic networks.

Another factor in using adult stem cells is that at least some types of them may function differently in males versus females. In one report estrogen-related effects were found to significantly affect how hematopoietic stem cells divided and renewed in female mice compared to those same types of stem cells in males [61, 62]. How much or if such gender-related differences might occur in humans is uncertain.

Despite these potential limitations, iPSCs might be particularly suitable for treating single-point genetic disorders. This technique has been used with some success in a mouse model involving replacing the gene associated with sickle cell anemia with a “normal” one [63]. For this type of therapy to be successful the genetically corrected cells derived from iPSCs must survive and preferably, if it is not associated with other risks such as development of cancer, increase their numbers [42]. A limiting factor for this or other uses of iPSCs is that it typically takes weeks to develop them from a person’s somatic cells—a delay that could become an issue depending on how quickly they are needed (e.g. to replace damaged tissues).

Recent reports suggest that techniques used to create iPSCs within a mouse rather than *in vitro* might actually produce cells that are totipotent rather than “merely” pluripotent [64, 65]. As described previously this means that they would not only be able to generate all the cell types that ESCs can but also the placental tissue that a blastocyst can. Whether these apparently totipotent cells could then be harvested and used to generate a complete animal as well as whether these findings could be applied to humans are questions that are still unanswered.

These results also raise the possibility of inducing a person’s cells to become iPSCs and subsequently differentiating without first removing them from the body. The obvious issue, however, is to make sure only the appropriate cells do this and only in the way desired, without forming the wrong, dysfunctional, or otherwise unwanted tissue instead of a healthy replacement. As in many other instances, the road extending from a proof-of-principle to safe, effective, routine therapy can be very long and challenging.

Another intriguing observation is that some mature, differentiated cells in the body can display some capability for self-renewal on their own under certain circumstances. Macrophages, a type of white blood cell, develop from hematopoietic stem cells. However, recent studies suggest they may also proliferate by dividing on their own if needed [66]. Hepatocytes, a kind of cell specific for the liver, also have the ability to do this, consistent with the liver’s much greater ability to regenerate itself compared to many other solid organs

such as the heart. These findings raise the possibility that some other types of mature, differentiated human cells that appear to have very limited (if any) ability to replicate and replace damaged ones may have more “talent” in this area than we currently give them credit for.

Finally, both iPSCs and ESCs might also prove useful in other areas of research devoted to learning how our bodies develop and function. For example, due to their pluripotency they can be used to better understand how human tissues and organs form during embryonic development [59]. This can include uncovering the details of how particular genes act individually or interact with others to create specific cells and modulate their functions. Learning the genetic, epigenetic, and environmental factors that make an individual iPSC or ESC differentiate into one particular kind of different cell rather than another could be of great importance in creating new tissues for therapeutic use.

13.3 Risks of Stem Cells

Both ESCs and iPSCs have two major intrinsic risks that currently hold back their use in human clinical trials [30]. Each one’s ability to form tissues derived from the three germ lines actually represents a two-edged sword. If the tissues created can grow and function normally, they could indeed help replace similar, damaged tissues in a person. However, ESCs and iPSCs also have the potential to develop in undesired ways. One is that they can form a type of tumor called a “teratoma.” In fact, the ability of a cell such as an ESC or iPSC to form a teratoma is a criterion used to verify that it is, in fact, pluripotent [64, 67].

Though uncommon, teratomas can occur spontaneously in a person, usually but not exclusively in an ovary or testis. They are thought to be typically present at birth, although their size at that time may range from very tiny to large [19]. Teratomas are characterized as containing tissue derived from at least two different germ layers. These tumors are typically “encapsulated” with a well-marked boundary of tissue, but they may contain strange mixtures of various ones, including hair and neurological tissue (ectodermal germ line) along with bone (mesodermal germ line). Teratomas are typically (although not always) “benign” in the medical sense of not spreading (“metastasizing”) to other parts of the body, but only growing locally in one part of it. Nonetheless, if a “benign” teratoma grows large enough, ruptures, or otherwise interferes with normal surrounding tissues (e.g. a teratoma located in the brain) it can cause significant secondary health problems. Less commonly teratomas

are “malignant” and can potentially spread to other parts of the body, similar to the behavior of many types of cancer.

Needless to say, developing a teratoma at a location where ESCs or iPSCs were used to “successfully” replace damaged tissue is highly undesirable. In some animal studies, transplanted tissue derived from ESCs did in fact produce teratomas, as well as other types of cancerous growths in the recipient [19]. This safety concern is one reason why there have been few human trials using ESCs, which have included one involving ESC-derived cells to treat a form of blindness and a since-discontinued one (based on financial rather than medical reasons) involving use of a certain kind of nervous system cell to treat spinal cord injuries [67, 68].

Another potential risk of iPSCs and ESCs (particularly the latter) is that, even if they do not form actual teratomas, they may be difficult to control when it comes to creating a particular type of desired cell or tissue rather than undesired ones. In this respect the pluripotency of iPSCs and ESCs might constitute a relative disadvantage. For them to be more useful for transplantation purposes the genetic and molecular mechanisms by which they maintain pluripotency might need to be identified and altered to restrict and direct their ability to differentiate.

In the case of iPSCs, the risk of developing tumors such as teratomas varies depending on the type of somatic cell used to generate them. This might be due to epigenetic factors and some genes in those cells being resistant to reprogramming [69]. It is possible, however, that if the genetic or other factors that lead to development of teratomas can be adequately identified, ESCs and iPSCs could be further modified to reduce this risk. However, more research is needed to address this issue.

Currently there have been no human clinical trials using iPSCs, although such studies have been proposed and may begin in the near future. Once again a primary consideration delaying such research is safety, based on the risk of iPSCs causing not only teratomas, but various types of cancer due to some of the methods used to create them. The original technique of using a retrovirus to introduce the four transcription factors needed to make an adult cell become pluripotent is associated with the retrovirus integrating itself into the host cell’s DNA. As mentioned in Chap. 12, the virus could do this at a critical location that either interferes with an important gene or with sections of the DNA that suppress tumor formation [40]. The iPSCs themselves could also have significant chromosomal abnormalities [70]. The net effect is that a differentiated cell produced from iPSCs could either not function properly or become cancerous.

Newer techniques to create potentially “safer” iPSCs have been used with varying degrees of success. A major advantage of using retroviruses, particu-

larly a lentivirus (see Chap. 12), to produce iPSCs is that they are considerably more efficient than some alternative methods for doing that, although their overall efficiency is still low. While some other types of retroviruses can introduce the “reprogramming” genes only into cells that are dividing, lentiviruses can also do this in cells that are not dividing [9]. However, lentiviruses too can integrate into a host cell’s DNA, thus also having the potential risk of turning it cancerous.

Other methods for producing iPSCs include using adenoviruses and a small RNA virus called the Sendai virus as vectors, as well as chemical modification, plasmids (small DNA molecules), and modified RNA [40, 42, 47, 69, 71, 72]. Combinations of particular molecules and fewer than the original four transcription factors (e.g. using only two, *Oct4* and *Klf4*) have also been used to create iPSCs [50]. One such study reported that iPSCs could be produced from mouse fibroblasts by treating the latter with only a “cocktail” of seven different molecules selected to induce pluripotency [41, 71]. This method made up to 0.2% of the fibroblasts convert to iPSCs, an efficiency that was comparable to techniques using transcription factors. How useful iPSCs produced by such non-integrating methods would be to treat human diseases has yet to be determined, however.

In some cases these alternative techniques might involve activating genes already present in a cell to make it become pluripotent rather than introducing new genes into it. The hope is that at least some of these other methods will be safer regarding the risk of developing cancer. Methods that do not result in integration of new genetic material into the cell’s DNA are considered safer than ones that do. However, it is still possible that they too might produce enough genetic and epigenetic changes to make the resulting iPSCs more susceptible to creating tumors rather than healthy differentiated cells. Moreover, most non-integrating methods are significantly less efficient at producing iPSCs than the original ones using retroviruses, although newer techniques may improve this [53]. In fact, recent reports indicate that a non-viral technique that modifies a single genetic factor could produce iPSCs with nearly 100% efficiency [73, 74].

For safety purposes, any method used to generate iPSCs would need to be determined to have a risk of teratoma formation that is as low as possible. Also, the differentiated cells produced from an individual patient’s iPSCs created by such a method would need to be tested or otherwise ascertained to be safe before transplantation.

As mentioned previously, a particular limitation of ESCs and tissues derived from them is that, if used for transplantation, they represent cells foreign to a person’s body. They are thus subject to being attacked and destroyed by the recipient’s immune system.

This problem might be partially ameliorated by methods discussed in detail later in this chapter that are similar to those used for organ and other types of transplantation. These include matching the tissue to be transplanted as closely as possible with those in a person's body based on major "antigens" (substances that the body recognizes as either foreign or part of it) and use of medications that suppress immune function. However, the latter can also potentially increase the odds of developing serious infections and cancer by itself. This problem might also be addressed by processing differentiated cells prior to transplantation to reduce incompatibilities with the host, although this would be less feasible for using actual organs derived from ESCs [68, 75].

Moreover, as mentioned previously the process of creating the iPSCs might modify them enough so that they are no longer identical to the host but might have enough changes to activate the immune system [68, 69, 75]. In some cases even adult stem cells obtained from a person's own body might, when used for transplantation, potentially evoke an immune response due to changes in them caused by the culturing process used to increase their numbers. Fortunately, however, this does not seem to be a significant risk in the case of mesenchymal stem cells [19].

Tumors and cancers can also have their own pools of stem cells [16]. In fact, it is now thought that tumors and other forms of cancer can originate from adult stem cells and partially differentiated (progenitor) cells derived from them that have, due to genetic mutations or other factors, become cancerous [2, 14, 19]. While stem cells are already self-renewing—a characteristic that cancer cells (unfortunately) also possess—progenitor cells must typically acquire this ability as part of a process that turns them cancerous. In particular, cancer stem cells have been identified as part of various types of leukemia, isolated from tumors involving the central nervous system, and found in cancers involving areas such as the breast, lung, liver, pancreas, colon, and prostate [2, 14, 76–78]. Some of the same transcription factors mentioned previously such as *Nanog* that keep cells pluripotent also appear to contribute to the ability of cancer stem cells to resist chemotherapy as well as increase their ability to spread and invade normal tissue [14].

Another potential source of cancer stem cells is from "mature" cancer cells that have undergone "reprogramming" and reverted to a stem cell-like state [14, 19, 78, 79]. This mechanism could also help renew the number of cancer stem cells beyond that produced by their own replication and increase a tumor's ability to produce additional mature cancer cells.

Just as the "healthy" type of stem cell present in many tissues can differentiate and create replacement cells, cancer stem cells can replenish the very unhealthy ones in a tumor. As with normal stem cells, cancer stem cells may constitute only a very small fraction of total cancer cells. For example, in

leukemia and multiple myeloma (cancers involving various types of blood cells), only 1–4 % and 0.001–1 % of malignant (cancerous) cells respectively were found to be cancer stem cells [4].

However, even a small number of cancer stem cells may act as a quiescent pool of cells that can produce new, mature cancer cells after the latter are killed during treatment. Most cells in a tumor are not involved at any given time in making it grow, and destroying them will cause the tumor to regress. However, the tumor can grow again due to division of cancer stem cells, which are typically resistant to chemotherapy, radiotherapy, and other treatments that destroy mature cancer cells [2, 80]. Such measures are generally most effective against dividing cells, and if the cancer stem cells are not active at a given time they would be expected to be less susceptible to those kinds of therapy. However, it is important to determine what other factors might contribute to this resistance to help improve treatment. These might include cancer stem cells possessing cellular systems designed to strongly resist apoptosis, efficiently repair their DNA, and/or remove toxic substances (including chemotherapeutic agents) from them [78].

Another concern is how sensitive normal stem cells are to injury from the chemotherapeutic agents and radiation doses used to treat cancer stem cells. If the former are more likely to be injured than the latter, those treatments could give the cancer stem cells a relative advantage for further growth. Also, while normal stem cells can protect themselves to a degree from cancerous mutations by becoming senescent or undergoing apoptosis when damaged by chemotherapy and/or radiation, cancer stem cells might do neither—again giving them a relative survival advantage. The challenge is to minimize damage to the “innocent bystander” healthy stem cells (as well as other normal cells) while targeting the cancerous variety [2, 14].

There is also a risk with obtaining stem cells from a person and increasing their numbers by culturing them outside the body before injecting them back into that individual for treatment. Stem cells themselves can become malignant after a sufficient number of divisions, and thus there is a chance that as they proliferate during culturing some might turn cancerous. It is possible that the “unnatural” environment of the culture itself could enhance this risk [19].

Even a person’s own mesenchymal stem cells may be “fooled” into actually promoting the growth of breast and other types of cancers [16]. They might also protect a tumor by reducing the body’s immune response to it. This raises the possibility that, if someone with an undiagnosed tumor is treated with a dose of mesenchymal stem cells, they could paradoxically make the person worse rather than better.

However, in other contexts ESCs and either neural or mesenchymal stem cells might be used to treat certain types of cancers that are resistant to other methods. For example, they have been used as “carriers” to deliver genes, therapeutic chemicals, and other substances to gliomas, a type of brain tumor with an overall poor prognosis that can occur in either children or adults [81, 82]. The gene(s) introduced into the tumor by this method may trigger them to self-destruct via apoptosis or kill them in other ways, as would any cytotoxic chemicals carried by the stem cells.

Another issue with iPSCs or any other technique using adult somatic cells such as fibroblasts is that these cells may have relative “deficiencies” that could be passed on to any differentiated cells produced by them. As described in Chap. 9, telomere shortening in fibroblasts and other somatic cells that typically undergo many replications normally limits how many times they can divide before becoming senescent. Fibroblasts derived from an adult that are used to generate iPSCs already have some degree of telomere shortening. The amount of shortening may vary, however, since some fibroblasts in the body may have undergone fewer replications than others.

Such considerations raise the questions of whether the healthy longevity of at least some types of differentiated tissue derived from iPSCs or adult stem cells will be shorter than normal or at least relative to tissue obtained from genetically “younger” ESCs, and whether the risk of malignant transformation might also be increased. A study involving ESCs obtained from mice bred to have innately short telomeres suggested that such ESCs had a reduced ability to become stably differentiated [83]. Again, whether or how much this observation might apply to adult somatic cells with reduced telomere length in humans is uncertain but suggests the need for further investigation.

13.4 Organ Transplantation

The concept of replacing a damaged or diseased organ with a healthy one is a simple one. However, it took nearly the entire twentieth century and into the current one to develop reasonably successful techniques for harvesting various types of donor organs, performing surgery, and reducing the risks of post-operative complications [84, 85]. The first successful corneal transplant using tissue from a human donor was performed in 1905 [85]. Initial (though unsuccessful) attempts at kidney transplantation occurred a year later in 1906, using organs obtained from either a goat or pig [84, 85]. Despite other attempts over the ensuing decades, the first successful human kidney transplant was not done until 1956, with the donated kidney coming from the

recipient's living, monozygotic ("identical") twin [84–86]. Current sources of kidneys for transplantation include both deceased and living donors.

Initial attempts at liver, lung, pancreas, and heart transplantations in the 1960s showed that the procedures could be successfully done from a surgical standpoint [87–90]. However, long-term survival for patients after these operations was initially poor, primarily due to rejection of the transplanted organs and infections associated with the "immunosuppressive" medications available at that time to reduce the risk of rejection. If the donated organ had sufficient differences in certain antigens from the recipient, particularly in key types such as the HLA ones described previously and the ABO blood groups,³ the recipient's body would recognize it as containing "foreign" molecules such as peptides,⁴ assume it was harmful (similar to the way it identifies "non-self" material in bacteria and other infectious agents), and activate its immune system to destroy the organ.

Unfortunately, on its own the human body is not "smart" enough to understand that, although the donated organ is not an original part of that individual's body, it is beneficial rather than dangerous like an infectious microorganism would be. The immune response that the body then generates, involving a variety of white blood cells and chemical pathways, is "non-selective"—a "shotgun" approach that does not distinguish between "innocent" targets like the transplanted organ and "guilty" ones such as harmful bacteria.

One way to work around this problem is via "tissue typing"—matching antigens as closely as possible between donor and recipient, particularly ones where differences would be most likely to trigger an immune response. These would include the HLA antigens, also known as the "major histocompatibility complex" (MHC) and, to a lesser extent, "minor histocompatibility antigens" (miH) [91]. This usually works best when the donors and recipient are close family members, who typically share more of the same antigens with each other than with non-related individuals. The degree of antigen "matching" is related to how close the family relationship is, with that in monozygotic

³ A particular individual's red blood cells may have both the A and B antigens (type AB); only the A antigen, with antibodies against the B antigen in the bloodstream (type A); only the B antigen, with antibodies against the A antigen in the bloodstream (type B); or neither the A nor the B antigen, and antibodies against both the A and B antigens in the bloodstream (type O). An immune response that destroys red blood cells will occur if a person receives a blood type containing an antigen he or she does not have. Thus, an individual with type AB blood can receive any other type of blood; type A can only receive type A or O; type B can only receive type B or O; and type O can only receive type O. There should also be compatibility regarding another red blood cell antigen, the "Rh factor." Thus, a person with "O+" blood is type O and has the Rh factor, while one who is "O–" is type O but lacks the Rh factor and can have antibodies against it. Thus, an O+ individual can safely receive blood from an O+ or O– donor, while someone with O– blood can only receive it from another O– individual.

⁴ Peptides are linear chains of amino acids that, if long enough, are called polypeptides. Proteins in turn consist of one or more chains of polypeptides that are biologically functional.

twins obviously being the closest. Depending on which antigens were inherited from each parent, siblings may or may not be close matches, a particular parent may share some but not all antigens with a child, and overall similarities decrease as relatives become more distant.

Another major method is to suppress the recipient's immune response so that it does not inappropriately "attack" the transplanted organ. Unfortunately, like the body's own immune response, all currently available immunosuppressive medications lack adequate selectivity in their effects. While they can successfully reduce the likelihood of rejecting a transplanted organ, they also decrease the ability of the recipient's immune system to appropriately recognize and deal with infections and malignant cells.

Infections are a common cause of complications following transplantation. This risk is generally highest soon after transplantation but remains significant thereafter, with the particular types of infections likely to occur varying depending on long it is after the transplant [92]. The overall risk of developing a malignancy is reported to be about 20% at 10 years following transplantation and nearly 30% after 20 years [93]. Skin and lip cancers are the most common type, representing about 36% of post-transplantation malignancies. Most such cancers, including squamous and basal cell carcinomas, are amenable to treatment. However, though less common than those types, the risk of developing a considerably more serious skin cancer, malignant melanoma, is also increased in transplant patients.

Likewise the incidence of malignancies associated with infections involving particular viruses, such as Kaposi's sarcoma and ones associated with "post-transplant lymphoproliferative disorder" (PTLD), is also increased. In PTLD the body produces too much of a certain kind of white blood cell (B lymphocytes) [94]. It is typically associated with new or prior infection with the Epstein-Barr virus, which causes infectious mononucleosis. PTLD can also result in fever, shortness of breath, and development of cancers such as lymphoma, particularly non-Hodgkins lymphoma.

Besides their intrinsic risk of suppressing both the beneficial and harmful effects of the recipient's immune system, immunosuppressive medications can have other significant side effects. For example, the good effects of one such medicine, cyclosporine, comes at the particular cost of toxic effects on the kidneys [84, 95]. Another one, sirolimus, can also harm the kidneys and have other adverse effects, such as impaired wound healing.

A post-transplantation complication that can lead to failure of the donated organ and death is "transplant arteriopathy [96]". This involves the recipient's immune system mounting a response against the blood vessels of the transplanted organ. Transplant arteriopathy can affect both large and small arteries. It can cause enough loss of blood supply to damage or destroy parts

of a transplanted organ, leading to deterioration of its overall function and perhaps ultimate failure.

When this vascular issue occurs in transplanted hearts it is called “cardiac allograft vasculopathy” (CAV) and represents a potentially fatal complication [96, 97]. CAV is associated with narrowing of the coronary arteries, reducing blood supply to the heart. However, it differs from “conventional” coronary artery disease (CAD) associated with atherosclerosis in several important ways. CAD is typically associated with localized blockages in one or more coronary arteries, and these lesions may be calcified. Conversely CAV can involve diffuse narrowing of very long lengths of all three coronary arteries, making those narrowed and typically non-calcified regions much less amenable to standard treatments such as coronary angioplasty and bypass surgery.

Moreover, the transplanted heart is at least partially “denervated”—that is, it does not have the usual connections with the body’s nervous system. Thus, while a person with significant CAD might feel chest pain (angina) when the heart does not get enough blood, the patient with a heart transplant and CAV may not feel angina due to lack of nerve fibers carrying sensations of pain. Long-term surveillance of post-cardiac transplantation patients can thus involve use of coronary angiography, which (as noted in prior chapters) is invasive and carries a small amount of risk itself. Moreover, as noted above, even if CAV is identified treatment options may be limited. Overall, the incidence of CAV has been reported to be 52% at 10 years following cardiac transplantation. It can be associated with myocardial infarction and arrhythmias that can cause “sudden death,” with the individual unexpectedly developing a lethal arrhythmia.

Thus, given these and other risks, there can be a fine line between balancing the overall beneficial and harmful affects of immunosuppressive therapy and transplantation itself. Nonetheless, the long-term success of many types of organ transplants is directly related to development of immunosuppressant agents that can do this reasonably well. Widespread use in particular of cyclosporine beginning in the early 1980s significantly decreased the rate of failure/rejection of transplanted organs (e.g. kidneys and hearts) and increased the overall long-term survival of organ recipients. Along with older medications such as azathioprine and corticosteroids, the availability of newer agents such as tacrolimus, mycophenolate mofetil, sirolimus (rapamycin), and many others to reduce the risk of acute rejection at the time of transplantation and for long-term use has led to increasing success in keeping transplant patients alive and healthy [84, 91, 95].

Transplantation procedures done mainly to improve quality rather than quantity of life raise other issues. For example, the first successful human face transplantation was performed in 2005. This technique involves using

various types of facial tissues from a deceased donor that might include skin, muscles, tongue, nose, lips, eyelids, scalp, and bones such as the mandible (“jaw bone”). Reports published early in 2014 describe up to 28 individuals receiving facial transplants worldwide, with an overall mortality rate of 11.5% in the first 26 patients [98, 99].

Individuals who receive face transplants must use immunosuppressive therapy for the rest of their lives. This increases their risk of developing the complications described previously, including various types of cancer and infections. Other risks of this procedure include those associated with the surgery itself and lifelong ones for acute skin rejection and graft-versus-host disease.

Overall, in select patients with very severe facial injuries, face transplantation potentially has cosmetic and often functional benefits for improved facial reconstruction compared to other methods. However, unlike a heart transplant that can, despite its risks, improve overall survival and quality of life, a face transplant is not only associated with its own increased morbidity but could decrease how long the recipient lives. Procedures such as a hand transplant, which can also have obvious functional benefits but is not essential for life, raise similar considerations. Such issues demonstrate the need to carefully weigh the risks and benefits of such a procedure, knowing that neither choice—to have the procedure or to decline it—is ideal and whichever one is made involves tradeoffs.

A major ongoing problem with organ transplantation is the scarcity of donors compared to prospective recipients. The “pool” of available organs has increased somewhat due to measures such as improved techniques for harvesting and preserving them for transplantation, as well as broadening the selection criteria to include donors who are older or had somewhat more “problematic” organs regarding the latter’s function and other characteristics. However, the rate of increase in the number of potential recipients continues to outpace that of donors.

A number of factors limit the number of donors. One is the relatively small number of individuals who both volunteer to be donors (or, if unable to give consent, have it given by family members) and qualify as donors of at least some organs based on age and other criteria. The latter include that the organ must be reasonably healthy; the donor should not have known infections, malignancies, or other issues that could impact the health of the transplanted organ and ultimately the recipient; there must be a suitable “match” based on antigen profiles, sometimes size, and other factors between donor and recipient; and many organs (particularly the heart) have a very narrow window of time between when it must be harvested and transplanted (see Chap. 7).

Another factor is the obvious one that, with certain exceptions such as kidney transplants, donors must be deceased prior to donating their organs, with

the heart being the most obvious example. This involves the critical issue of deciding when a donor actually *is* dead, which must be determined by strict medical and ethical criteria. It could also potentially involve heinous acts such as stealing organs from someone who is still alive or deliberately killing a person to obtain them.

As mentioned earlier, science fiction-related examples of using unwilling donors include Robin Cook's *Coma* (1977) and some of Larry Niven's "Known Space" stories. In the latter works the mismatch between supply and demand for organs is "solved" by either punishing individuals found guilty (with reduced regard for whether they are actually innocent instead) of what would now be considered trivial "crimes" with the death penalty, or by simply stealing them from victims ("organlegging"). However, the key practical problem with a society in which everyone (or at least the majority of the population) is at significant risk of being an involuntary donor/victim or recipient is that the net benefit/risk may be comparable for everyone, reducing if not eliminating the value of implementing of such an organ procurement system.⁵ Also, simply following current standard medical procedures for organ tracking (e.g. it is not standard operating procedure to purchase organs via online auctions or other poorly documented sources) and ethical guidelines should curb (but unfortunately, given enough individuals lacking such ethical principles, certainly not entirely eliminate) widespread use of the organlegging technique.⁶

Besides informed consent for the procedure, organ transplantation for "vital" organs such as the heart requires that the patient be "brain dead." This can be defined as the "complete and irreversible cessation of all brain and brainstem function [100]". Using this as the "standard" for death replaces earlier concepts that death occurred when the heart stopped beating and breathing ceased. Current medical treatments include cardiac defibrillators, pacemakers, and medications that can restore what would otherwise be a fatal arrhythmia. These and other methods such as mechanical ventilators can support a person's cardiopulmonary system well enough to keep the rest of the body's remaining organs functioning even when brain death has occurred. An all too common scenario is that of a young adult who has suffered severe head trauma or another cause of isolated brain/brainstem injury that left the rest of the body reasonably intact and functional.⁷

⁵ However, as a *reductio ad absurdum* thought experiment the concept as Niven portrays it is indeed very powerful.

⁶ The overall less fatal procedure of "buying" an organ such as a kidney from a living donor also has its own serious ethical issues.

⁷ Part of the evaluation to assess whether or not an individual who has been declared brain dead is a suitable donor for a heart transplant is to confirm that the heart is functioning adequately. During my career

A variety of guidelines have been developed to make the diagnosis of brain death [101]. Key points include ascertaining that no reversible reasons for loss of brain activity are present (e.g. medications or hypothermia—see Chap. 7), the presence of multiple specific findings on a neurological examination (including but not limited to criteria for coma and absence of many types of reflexes), and performance of tests designed to assess brain activity and/or blood flow. The challenge is to ensure that the best possible criteria for evaluating and confirming brain death, with complete and irreversible loss of brain and brainstem function, are used and applied properly. In particular, brain death must be distinguished from other diagnoses such as a persistent vegetative state (a condition associated with severe brain damage and reduced consciousness) that have at least the potential to improve. Given the critical ethical principle of not removing vital organs from an individual who is not truly brain dead, it is essential that current standards for that diagnosis continue to be discussed and refined as needed based on further reviews and findings.⁸

Another method to increase the supply of donor organs is to use ones from animals—“xenotransplantation.” As noted previously, some early attempts at organ transplantation involved taking them from goats, pigs, etc. Many organs in pigs are similar in size to those of humans, and they can be obtained in great abundance. In fact, specially treated heart valves derived from pigs or from the pericardium (the thin sac of tissue enclosing the heart) of bovines are used to create certain kinds of artificial heart valves for humans.

However, there are at least three major biological issues for using animals as organ donors in humans. Some animal tissues (particularly those from pigs) can be genetically modified to produce certain types of human proteins that “fool” part of the recipient’s immune system into “thinking” they are not foreign material. Unfortunately methods have not yet developed to protect those porcine or other organs from other components of the human immune systems and prevent rejection.

Also, although pig organs may be similar in size to the human versions, there are still major differences in their physiology, proteins and other constituents, cell function, etc. Cumulatively these factors could significantly reduce the ability of the transplanted organ to function and survive inside its new human host.

I have had to interpret echocardiograms (ultrasound studies of the heart) to make that determination. If I found that heart function was preserved, the organ could then be harvested. But even with the donor truly brain dead, with no hope of recovery, and knowing the heart may save someone else’s life, the tragedy and finality of death in that situation is still inescapable.

⁸ The plot of Robert J. Sawyer’s *The Terminal Experiment* (1995) addresses this issue in a science fiction context, raising the question of when exactly death occurs.

A third, less well-established risk is that of “zoonotic infection.” This means that a particular microorganism (e.g. a virus) that has infected the animal’s organ but normally does not infect humans could, following transplantation, potentially cause an infection in the human recipient. For example, certain porcine retroviruses might be able to do this.⁹

Another approach to increase the supply of transplantable organs is to actually grow them in some way. An idea presented in works like the movies *Parts: The Clonus Horror* (1979) and *The Island* (2005) is that human clones would be created to serve specifically as organ donors for the “original” versions of the clones. The rationale for doing this is to ensure that tissue-matched organs would be available to possible recipients should they be needed.

Besides the obvious ethical issues involved, the practical difficulties involved with this method would be enormous. For example, it assumes that human clones could be created and develop normally (assumptions that, as described in Chap. 10, are by no means certain); that the clones would not become ill, die, or be at an inappropriate age (e.g. too young) before they were needed to become donors; that the potential recipient would eventually need an organ transplant and not die from other health problems for which an organ transplant would not be helpful; that someone would pay the great costs involved with creating, feeding, providing healthcare, etc. and otherwise supporting the clone before it might be needed to serve as a donor; that the clones and others will not learn of this arrangement and object to it (something that, as expected, the above movies do show happening); and so on. In short, this method to procure organs for transplantation is, besides being grossly unethical, tremendously complex and costly for the proposed “return on investment.”

Fortunately considerably “simpler” methods are being investigated for creating new organs (and certain tissues) for transplantation. 3D printing techniques may be applied to create an inorganic structure in an appropriate shape (scaffold), such as to replace part of a bone in a person’s body [102]. Osteoblasts and other bone cells could then be introduced to the scaffold where they could proliferate and help form part of a new bone. After implantation of this bone-like structure into a person’s body, that individual’s own cells could also be stimulated by its presence to promote further growth and healing. Similar concepts underlie methods used to create other artificial structures such as outer ears (auricles) [103, 104].

⁹ Other viruses thought to have “crossed over” from non-human animals to humans include the HIV virus (a retrovirus), the H5N1 strain of avian influenza (very loosely termed the “bird flu”), and the MERS (Middle East respiratory syndrome) coronavirus.

While still an investigational technique, a small number of individuals have received transplants of bioengineered tracheas¹⁰ that have been seeded using their own stem cells [105]. In this case the scaffold for the stem cells can be provided by an animal (e.g. pig) trachea that has been “decellularized,” removing its own cells except for the tissue (“extracellular matrix”) that gives it structure. In animal models other tissues and at least part of whole organs have been created using similar methods with various types of cells, including lungs, liver, and the heart [106–109]. Mammary gland cells and prostate cells have also been generated from individual stem cells and used in animal transplantation studies to help regrow those organs without requiring a scaffold.

Overall, such techniques for growing tissues and organs for transplantation are at varying stages of development. Tissues with a relatively simple structure and composition such as the cartilage in outer ears or parts of certain bones may be particularly amenable to scaffolding and similar (e.g. 3D printing) methods. Liver tissue, with its intrinsic ability to regenerate, might also prove amenable to production of grafts using a scaffold matrix.

However, the heart has very complex anatomic, physiological, mechanical, and electrical properties that leave little room for error. While animal studies have taken the first steps in creating at least a miniature version of a bioartificial heart, [108] this is a far cry from using a person’s own cells to build a fully functional and healthy replacement should anything bad happen to the original. On the other hand, using stem cells to help repair a damaged heart might be easier. A recently described technique used yet another type of stem cell, “parthenogenetic stem cells,” to create myocardiocytes [110]. In one study this type of stem cell was derived from mouse oocytes that were stimulated via chemicals to start dividing and developing. This produced pluripotent stem cells with some characteristics similar to ESCs, but unlike the latter they could not develop into a complete new organism.

The parthenogenetic stem cells differentiated into various cells types including myocardiocytes, which were then transplanted into the hearts of the mice that donated the oocytes and others that were compatible matches regarding important antigens. Those transplanted myocardiocytes were reported to both function well and not be associated with rejection. If similar techniques could be applied in humans, this variety of stem cell might serve as a source of (potentially banked) tissue for repairing hearts damaged by a myocardial infarction or other event.

As a science fiction connection to the history of heart transplantation, there actually have been and still are individuals with two hearts who were not from

¹⁰ As described in Chap. 1, the trachea is the “tube” (located primarily in the neck) that connects the pharynx, the upper part of the “windpipe,” to the bronchial tubes of the lungs.

Gallifrey. The first “heterotopic” heart transplant was done in 1974 by Dr. Christian Barnard, who performed the first “orthotopic” operation to replace one human heart with another in 1967. In a heterotopic operation a person’s original, damaged heart is left in place and a new, donor heart is implanted into the right side of the chest. This procedure can involve connecting the new heart to the old one in such a way that it either assists only the left side of the original heart or both the right and left sided sets of chambers (see Chap. 1).

Though done far less frequently than orthotopic transplants, the heterotopic technique is still an option in individuals meeting certain criteria. These can include significantly increased pressures in the pulmonary (lung) blood vessels; if the available donor heart is considered marginal for being able to take over a person’s entire circulation; or if it is not an ideal match to the recipient regarding HLA or other antigen typing and thus has an increased risk of rejection. An obvious advantage is that, if the donor heart is rejected, the patient still has the original heart present. A disadvantage is that the second heart can develop its own problems such as potentially serious arrhythmias and blood clots forming inside it. However, in selected patients it can be a lifesaving treatment [111].

13.5 The Bottom Line

The concepts of stem cell therapy/regenerative medicine and organ transplantation can serve as the primary focus of a science fiction work (e.g. *The Island*). Or it can be just element in a plot, such as in Lois McMaster Bujold’s *Memory* (1996) which opens with the novel’s protagonist recovering from major tissue and organ transplantation procedures. As will be discussed in the next chapter, two episodes of *Star Trek: The Next Generation* deal specifically with the artificial heart Captain Picard received after his original, biological one was damaged.

Current research in these fields is going in many different directions. The pace at which particular issues will be addressed—what types of stem cells will prove most useful and safe for tissue/organ repair or replacement, dealing with the complex immunological problems with transplantation, growing whole new organs—is uncertain. It is also unclear what the theoretical and practical limits of various avenues of research might be, such as whether the risks of using stem cells might (at least in some circumstances) outweigh their benefits or if complex organs such as the heart (much less the brain!) can actually be created at a level of function and safety as well as in numbers suitable for extensive clinical use.

Science fiction, unfettered by many real-world considerations of the scientific difficulties involved, can use ideas involving regenerative medicine and organ transplantation in many ways and settings. However, extending them too far for story purposes can reduce the work's realism regarding other considerations, as mentioned previously regarding *Parts: The Clonus Horror*. If such techniques do become feasible, all of their ramifications—including how practical they are to perform, how much they cost, how long it takes to perform them, what resources are needed to use them, etc.—should be taken into account if only implicitly.

Likewise, using organ transplantation or advanced techniques for tissue repair using stem cells or other means to help explain why a character is 200 years old is also suspect. While such methods can certainly help an individual (e.g. one requiring a heart transplant) live longer than would otherwise be possible, they might have at most little impact on extending maximal lifespan beyond the known (at least so far) upper limit of about 122 years. As noted in Chap. 9, aging is a process that involves the whole body and is in a fundamental sense different from “disease.” While it certainly may act as a stopgap measure to prolong life, replacing a liver or kidney will do nothing to restore an elderly individual's immune system, clear that person's cells of accumulated DNA mutation and damage, prevent degeneration of the brain, etc. Other methods will have to be developed (if they can be) to address such issues. The net result is that many, many “medical miracles” will likely need to be developed (once again, if they actually can) in many, many different and distinct areas (genetic engineering, nanotechnology, use of stems cells and organ transplantation, etc.) to create a biologically youthful 200-year old human with intact cerebral function.

Many other medical issues might also be skirted over in science fiction with a similar loss of realism. For example, it takes a certain amount of time for cells to divide and grow. Thus, if a character receives a new liver grown from his or her cells, if those cells were obtained today there would simply not be enough time to have that organ be ready for transplantation tomorrow.

For example, H. L. Gold's short story “No Charge for Alterations” (1953) depicts a future physician literally remolding a “patient's” body like a sculpture as well as altering her personality. The physical alterations involve instantaneous plastic surgery that includes wholesale removal/reshaping of skin, bone, etc., and addition of new “cells” and “synthetic tissue.” They are vividly portrayed by sentences such as “The flesh fled from the cathode and chased after the anode as he broadened the fine nose, thickened the mobile lips, squared the slender jaw and drew out carefully the delicately arched orbital ridges.” However, the process described grossly underestimates the complexity of those parts of the body, the myriad types and organization of its cells and

tissues, the body's ability to heal, etc., and overall has at best a tenuous relation with actual human biology.

Similarly F. L. Wallace's "Tangle Hold" (1953) opens with the main character recovering from what is essentially a total "skin transplant." He had suffered burns from scalding high-pressure water and other trauma so severe and extensive that, he is told, "...every square inch of your skin is now synthetic." His treatment is said to involve peeling off burned parts, fitting the synthetic skin, then spraying on a bandage, after which "New cells form with this synthetic substance as the matrix." However, as noted in Chaps. 1 and 2, human skin has multiple layers, many kinds of different cells, a complex pattern of nerves and blood vessels, and performs many more functions (e.g. heat regulation) than just protecting our internal anatomy. How the synthetic skin would allow something as simple but essential as sweating is not addressed. Likewise, burns sufficient to "require" replacing a person's entire skin would also presumably involve at least some fourth-degree burns to underlying tissues, including the most vulnerable ones such as those of the penis and scrotum. Likewise the eyelids would offer little protection to the eyes in that situation, but there is no description of the latter being "boiled."

However, the focus in both stories is clearly not on medical realism but exploring the ramifications *if* it were possible to do those essentially impossible things. That "if" *is* a valid idea to explore in a story, even at the cost of making it science fantasy instead of science fiction.

Moreover, in the real world major operations place great stress on the human body. They can also be associated with many potential operative and postoperative complications. Unless one invokes "magical" methods such as nanotechnology far in advance of current capabilities, a character will simply not be anywhere near fully recovered the day after a heart transplant. Our bodies also take a significant amount of time to heal and recuperate long after surgery. In fact, an individual may never recover completely in some ways and can have delayed complications associated with scarring, infections, etc.

In short, for procedures such as stem cell therapies or advanced transplantation techniques to become "routine" they must (among other things) have an overall high initial success rate as well as a sufficiently low rate of immediate and delayed complications and adverse effects. A futuristic science fiction story can take these things for granted, but ideally it should not ignore fundamental principles of biology (e.g. rate of cell division) or the basic limitations of the "standard" human body. And even if this idea is not directly reflected in a story, hopefully this chapter will help the writer understand how far medical science has yet to go to bring these techniques to their imagined (and possibly real) potential.

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