to facilitate this process by developing technologies involving the expertise of biologists, chemists, engineers, and mathematicians. The high-throughput machine, DNA sequencer, was crucial to the success of the genome project. GRAIL (Gene Recognition and Assembly Internet Link) was one of the initial computer programs for identifying genes in DNA and DNA sequence analysis. The automation process of DNA sequencing led to cutting costs and hastening the DNA sequencing process.

2.5 STEM CELLS AND THEIR APPLICATIONS

Stem cells are unspecialized cells having the ability of self-renewal through cell division for long period. These stem cells have the potential to develop into specialized cells such as blood cells, muscle cells, neurons, myocytes, bone cells, hepatocytes etc.

Importance of stem cells

Stem cells can replicate indefinitely so they are serving as internal repair system for body to replace dead or damaged cells.

When a stem cell divides, one of the daughter cells has to remain unspecialized (like a parent stem cell) and the another daughter cell has become specialized cell type such as brain cell, blood cell under certain physiological condition (Fig. 2.9).

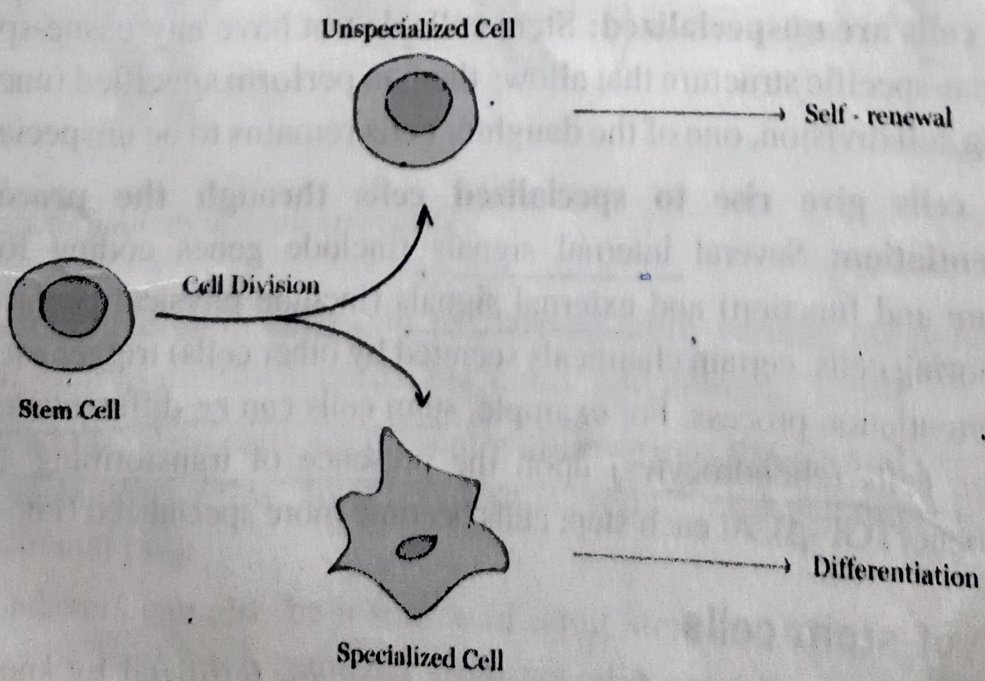


Fig. 2.9 *Unique properties of stem cells*

This is a promising area to know how an organism develops from a single cell with different types of potential properties. Since stem cells are having unique regenerative abilities, they offer new potentials for treatment of various diseases those results from dysfunction of a single type of cell.

Unique properties of stem cells

Stem cells are having three unique properties (Fig. 2.10). They are:

1. **Self-renewal:** Stem cells are immortal, unlimited in number and are capable of replicating/proliferating for a long period. They have potential to divide and maintain long term self-renewal.

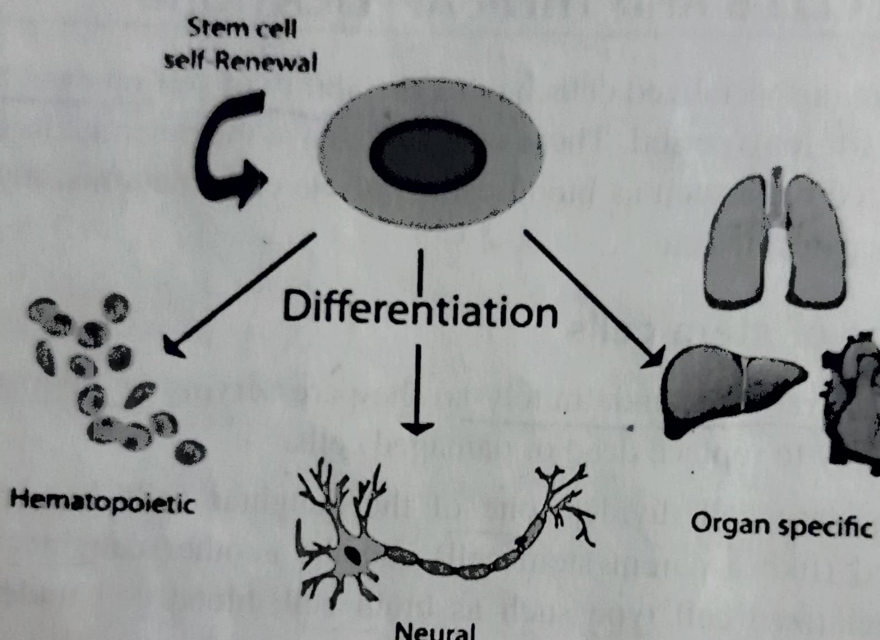


Fig. 2.10 *Differentiation of stem cells into other types of cells*

2. **Stem cells are unspecialized:** Stem cells do not have any tissue-specific or organ-specific structure that allows them to perform specified functions. During cell division, one of the daughter cells remains to be unspecialized.
3. **Stem cells give rise to specialized cells through the process of differentiation:** Several internal signals (include genes coding for cell structure and function) and external signals (include physical contact with neighboring cells, certain chemicals secreted by other cells) trigger each step of differentiation process. For example, stem cells can be differentiated into cartilage cells (chondrocytes) upon the presence of transforming growth factor-beta (TGF-β). At each step, cells become more specialized (Fig. 2.10).

Sources of stem cells

Embryonic stem cells: In embryogenesis, eggs are fertilized by sperm *in vitro* which is known as *in vitro* fertilization. As a result, zygote is developed which undergoes series of cell division, and produces blastocyst. In early blastocyst stage (5 -7 days), a group of approximately 30 cells called inner cell mass (ICM) is surrounded by an outer layer. The outer layer is called as trophoblast which provides nutrient to the embryo and develops into a large part of the placenta. ICM can give rise to all types of cells or tissues except trophoblast.

Adult stem cells: Adult stem cells are tissue-specific, undifferentiated cells found in differentiated tissues or organs including brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testes (Fig. 2.11). Their main role is to play in tissue repair and tissue maintenance.

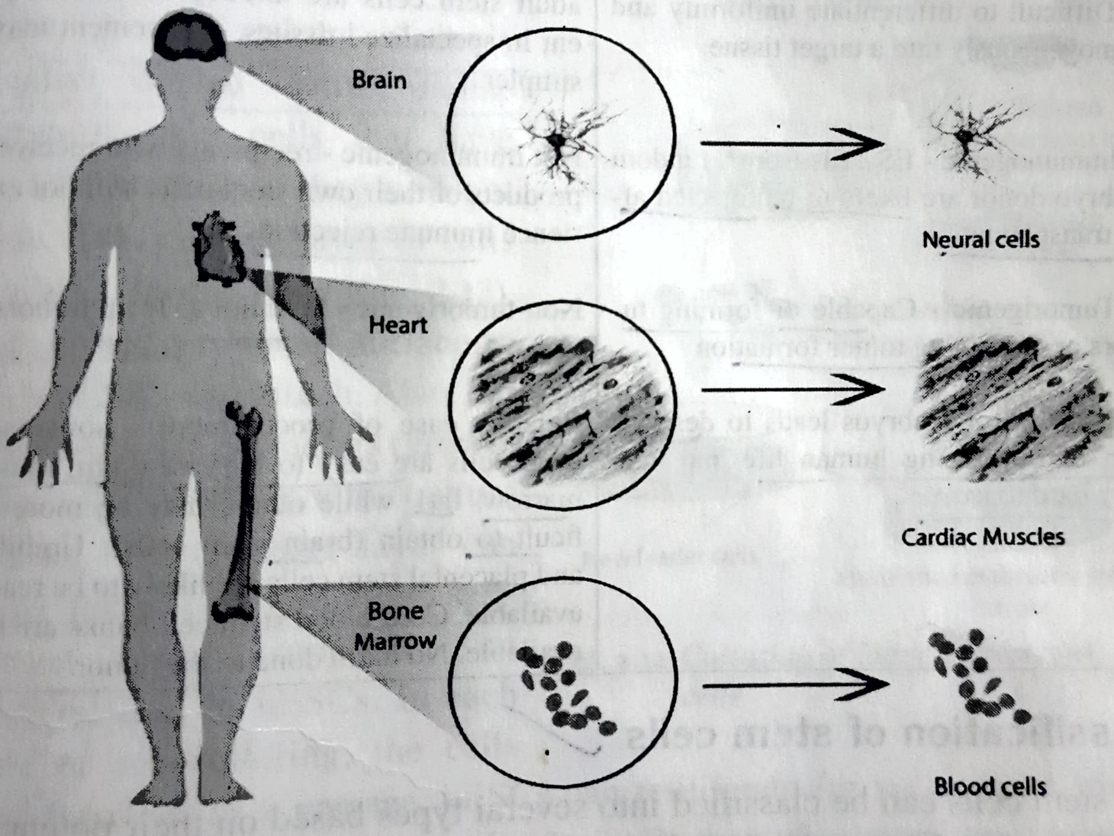


Fig. 2.11 *Sources of adult stem cells*

Adult type stem cells can be derived from various pregnancy-related tissues such as umbilical cords, placentas, and amniotic fluids.

In adults, stem cells are present within various tissues and organ systems such as bone marrow, liver, epidermis, retina, skeletal muscle, intestine, brain, dental pulp.

Cadavers can also be a source of adult stem cells. For example, neural stem cells have been removed from specific areas in post-mortem human brains as late as 20 hours following death.

Differentiation between embryonic and adult stem cells

Embryonic stem (ES) cells	Adult stem cells
1. Flexible- i.e., ES cells appear to have the potential to make almost all types of cells except trophoblast.	Less flexible- i.e., difficult to reprogram to form other tissue types.
2. Immortal- one ES cell line can potentially provide an endless supply of cells with defined characteristics.	Mortal with finite life time when cultured.
3. Availability - embryos can be obtained from in vitro fertilization and nuclear transplantation source.	Limited quantity - can sometimes be difficult to purify and obtain in large numbers.
4. Difficult to differentiate uniformly and homogeneously into a target tissue.	adult stem cells are already somewhat present in specialized tissues, inducement may be simpler.
5. Immunogenic - ES cells from a random embryo donor are likely to be rejected after transplantation.	Not immunogenic - recipients who receive the products of their own stem cells will not experience immune rejection.
6. Tumorigenic - Capable of forming tumors or promoting tumor formation	Non-tumorigenic - tend not to form tumors.
7. Isolation of embryos leads to destruction of developing human life and also very hard to isolate	Relative ease of procurement - some adult stem cells are easy to harvest (skin, muscle, marrow, fat), while others may be more difficult to obtain (brain stem cells). Umbilical and placental stem cells are likely to be readily available. Cord blood stem cell banks are now available. No harm done to the donor.

Classification of stem cells

The stem cells can be classified into several types based on their potency or plasticity. Potency or plasticity can be defined as the ability of the stem cell from one tissue to generate the specialized cell type(s) of another tissue.

Unipotent stem cells can form only one type of specialized cell type. For example, brain stem cells differentiate into only brain cells.

Multipotent stem cells can form multiple types of cells. For example, mesenchymal stem cells derived from bone marrow can differentiate into osteoblasts, adipocytes, chondrocytes, myocytes, and neuron-like cells.

Pluripotent stem cells can differentiate into almost all types of cell lineages. For example, cells (ICM) from blastocyst can differentiate into three germ cell layers (ectoderm, mesoderm and endoderm) but do not contribute for trophoblast.

Totipotent stem cells can differentiate into all cell types including cells of the trophoectoderm lineage. For example, fertilized egg and early cleavage stage of blastomeres.

Human embryonic stem cell isolation and culturing

Cell culture is a process of growing cells in the laboratory conditions. For generating human embryonic stem cells (hESCs), cells (ICM) from blastocyst are transferred into a culture dish containing a nutrient broth known as **culture medium.** The cells attach, proliferate and spread on the surface of the dish. The inner surface of the culture dish is typically coated with mouse embryonic skin cells that have been UV treated so they will not divide. This coating layer of cells is called a **feeder layer (Fig. 2.12),** which provides a sticky surface to which hESCs can attach. Also, the feeder cells release factors (growth factors, cytokines) into the culture medium. Thus, feeder layer can act as niche for promoting self-renewal or suppress of differentiation of hESCs. In each cycle of sub-culturing, the cells are referred to as a **passage**. hESCs can proliferate for six or more months without differentiation but can also maintain their pluripotent properties.

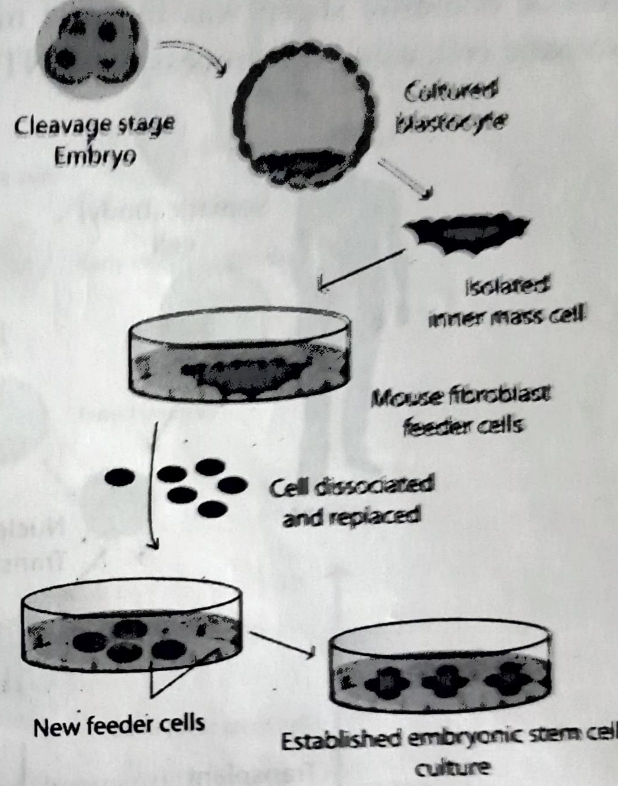
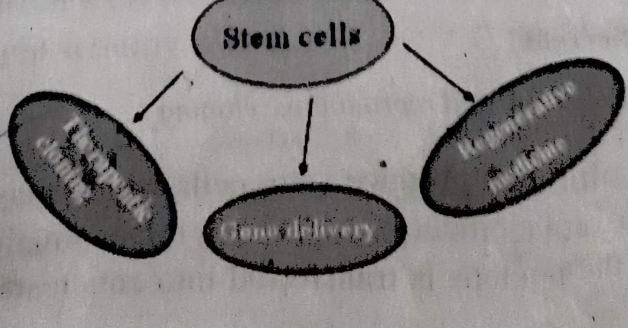


Fig. 2.12 *Culturing of human embryonic stem cells*

Applications:

Stem cell technology has a broad range of applications; here is an overview:



Therapeutic cloning

Dolly Sheep clone

Somatic cell nuclear transfer (SCNT) involves extracting the nucleus of a cell and putting the nucleus into an egg which has been enucleated. Then, the egg is allowed to divide and grow. In therapeutic cloning, the growihg egg is used as a source of stem cells, which are undifferentiated cells that can grow into a wide variety of different types of cells. In reproductive cloning, the egg is implanted into surrogate mother to grow a baby (Fig. 2.13). Dolly, a first female domestic sheep was the first mammal to be cloned from an adult somatic cell, using the process of SCNT.

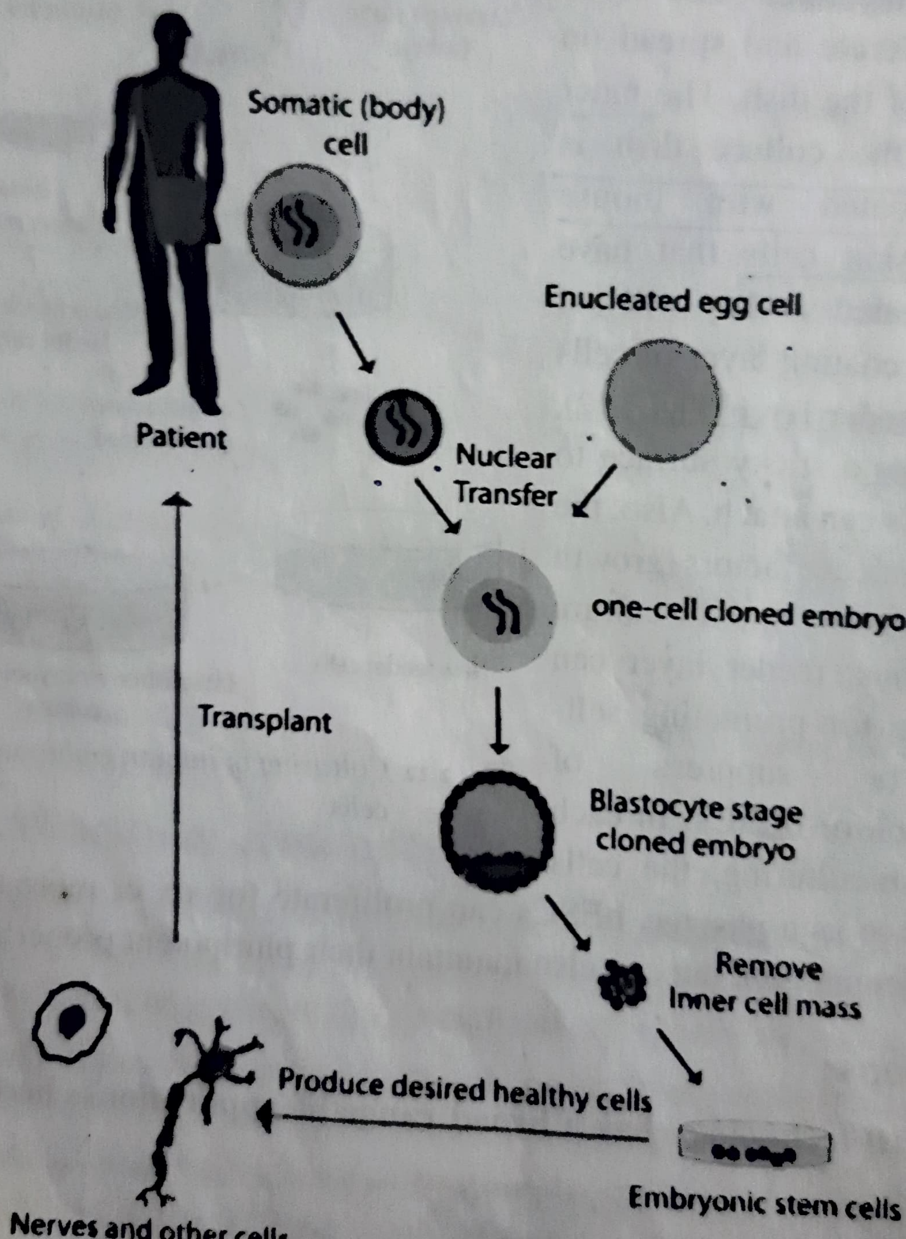


Fig. 2.13 *Therapeutic and reproductive cloning*

Therapeutic cloning can produce stem cells which may be used to treat various disorders or replacement of organs. In this, somatic cell is obtained from a patient and the nucleus is transferred into enucleated egg cell which will develop into an embryo. At blastocyst stage of the embryo, the inner cell mass is isolated and cultured to differentiate into particular cell type which will be replaced into the patients as a transplant (Fig. 2.14).

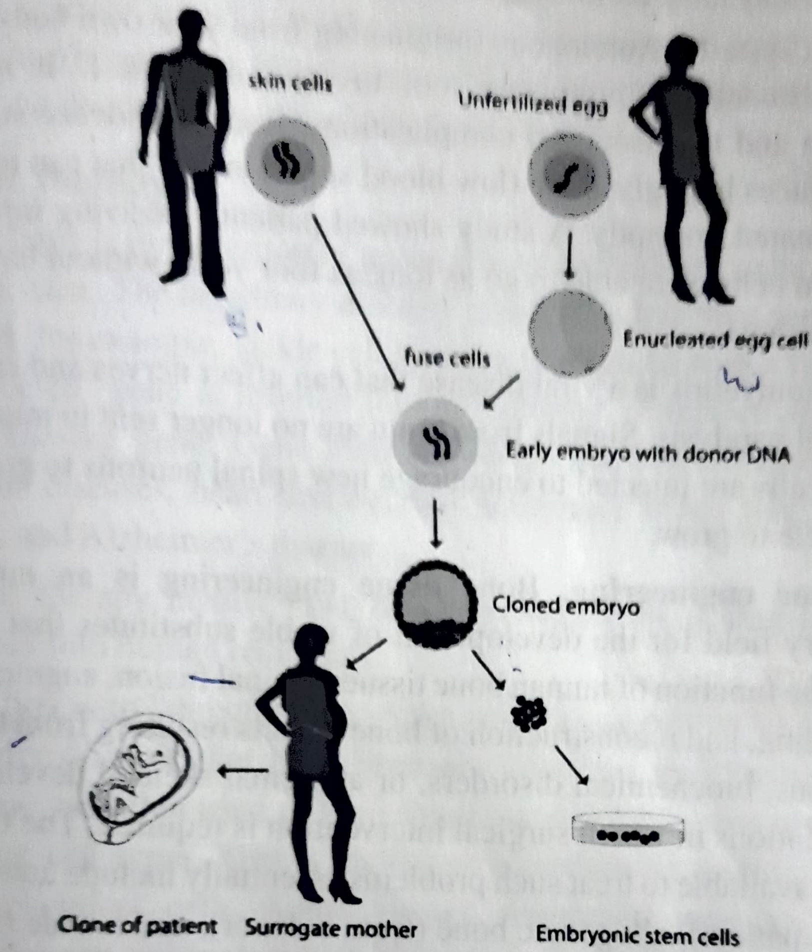


Fig. 2.14 *Theoretical concept of therapeutic cloning*

Regenerative medicine

Regenerative medicine is the process of creating functional tissues to repair or replace tissue or organ function lost due to damage, or congenital defects. This field holds the promise of regenerating damaged tissues and organs in the body by stimulating previously irreparable organs to heal themselves. Regenerative medicine also empowers scientists to grow tissues and organs in the laboratory and safely implant them when the body cannot heal itself.

Benefits of Stem Cell Research in curing diseases: Stem cell research can potentially help treating a range of medical problems. It could lead us closer to cure:

Parkinson's disease: A degenerative disorder of the central nervous system due to the lack of a brain chemical called dopamine. To cure this disease, the patients are injected with stem cells to multiply nerve cells that release dopamine.

Muscular dystrophy is a group of inherited disorders that involve muscle weakness and loss of muscle tissue, which get worse over time due to weakened heart and lung muscles. Patients are given injections of healthy stem cells and they are able to walk faster.

Diabetes (Type 1): Autologous (originating from your own body) stem cell therapy becomes a promising tool to treat diabetes I. It reduces hyperglycemia and its associated complications. Recent evidence suggests that it also reduces hypoglycemic (low blood sugar) events that can result in death if not treated promptly. A study showed patients receiving injections with adult stem cells were able to go as long as four years without having to rely on insulin shots.

Polio: Poliomyelitis is a viral disease that can affect nerves and can lead to partial or full paralysis. Signals from brain are no longer sent to muscles in the leg. Stem cells are injected to encourage new spinal neurons to grow and help new muscle to grow.

Bone tissue engineering: Bone tissue engineering is an emerging interdisciplinary field for the development of viable substitutes that restore and maintain the function of human bone tissues. Spinal fusion, augmentation of fracture healing, and reconstruction of bone defects resulting from trauma, tumor, infections, biochemical disorders, or abnormal skeletal development are clinical situations in which surgical intervention is required. The types of graft materials available to treat such problems essentially include autologous bone (from the patient), allogeneic bone (from a donor), and a wide range of natural or synthetic biomaterials such as metals, ceramics, polymers, and composites. In one approach, mesenchymal stem cells are seeded on scaffolds (that provide structure and shape) along with signaling molecules. The goal is for the cells to attach to the scaffold, and thus resulting proliferation and

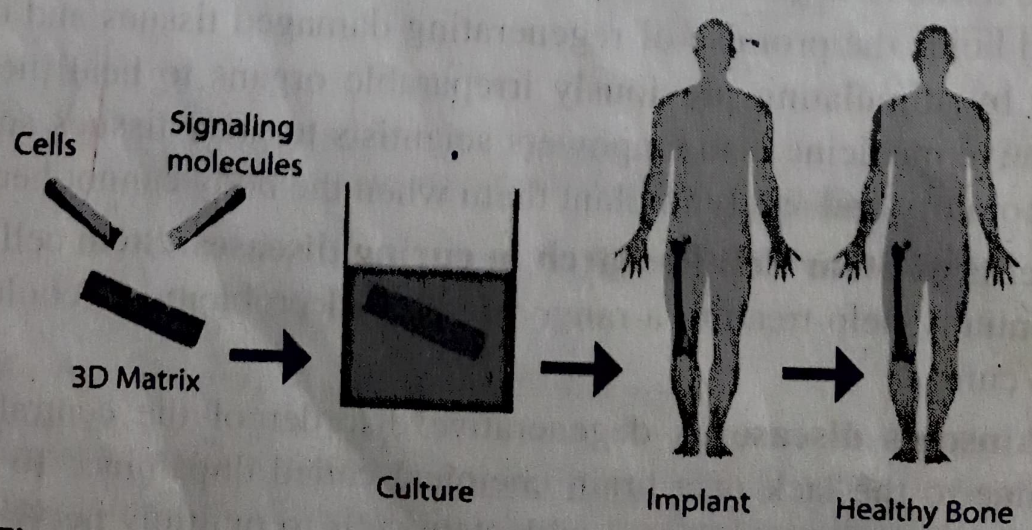


Fig. 2.15 Scaffolds guided ...

differentiation of cells into normal, healthy bone. When bone grows, the scaffold degrades. At one stage there will be only bone and no more scaffold materials. The signaling molecules [for example, bone morphogenetic proteins (BMPs)] can also be adhered to the scaffold or incorporated directly into the scaffold material. BMPs promote mesenchymal stem cells towards osteoblasts (bone cells). Finally the scaffolds are implanted into the defect to induce and direct the growth of new bone (Fig. 2.15).

2. Gene therapy

Gene therapy is the insertion of genes into an individual's cells and tissues to treat a disease. The hereditary diseases in which a defective gene (nucleotide mutation, for example, sickle cell anemia) is replaced with a functional one. A "corrected" gene is inserted into the genome to replace an "abnormal," disease-causing gene. Gene therapy clinical trials are focused on cancer, infectious diseases, heart disease, and inadequate blood flow to the limbs, arthritis, and Alzheimer's disease.

Gene therapy involves two major strategies for delivering therapeutic transgenes into human recipients:

1. The first is to "directly" infuse the gene into a person by viral mediated transfer. Viruses that have been altered to prevent them from causing disease are often used as the vehicle for delivering the gene into certain human cell types. This is similar to transduction process how ordinary viruses infect cells. Some viruses commonly used as gene-delivery vehicles can only infect cells that are actively dividing. This limits their usefulness in treating diseases of the heart or brain, because these organs are largely composed of non-dividing cells.
2. The second strategy involves the use of living cells to deliver therapeutic transgenes into the body. In this method, the delivery cells often a type of stem cell, a lymphocyte, or a fibroblast, are removed from the body, and the therapeutic transgene is introduced into them via the vehicles. The genetically modified cells are tested and then allowed to grow and multiply and, finally, are infused back into the patient.

density of stem cells in cell growth dishes determine further differentiation of these cells. To give a uniform density, electronic control of seeding density of stem cells have been achieved using thin electrode films of poly(3,4-ethylenedioxythiophene) (PEDOT): Tosylate.

Conclusions

Stem cells can now be artificially grown and transformed into specialized cell types with characteristics consistent with cells of various tissues. They can be taken from a variety of sources, including umbilical cord and bone marrow. Embryonic stem cell lines and autologous embryonic stem cells generated through therapeutic cloning have been proposed as promising candidates for future therapies. Adult stem cells are currently tested and used in medical therapies including tissue engineering. There are research and medical ethics associated with rules and regulations to be followed when stem cells are used for biomedical applications in human.

Chapter highlights

- The varieties of life on the Earth forming biodiversity are critical to sustenance of life on this planet. It can be genetic diversity, species diversity, and ecosystems diversity. Biodiversity is facing challenges and losses due to human population growth and their life style that may interfere and harm human way of life.
- All living and non-living beings are made up of atoms which then form molecules. The atoms within molecules are held by chemical bonds that are either weak or strong.
- In all living organisms, biochemical reactions called as metabolism occur that are essential for the survival of cells, structure, transport, cell signaling, cell division, etc.. Metabolism involves both anabolism and catabolism of biomacromolecules such as carbohydrates, lipids, proteins, and nucleic acids.
- Protein synthesis involves synthesis of proteins by the cells through the multi-step processes of transcription, post-transcriptional modification, translation and post-translational modifications.
- DNA replication is followed by messenger RNA (mRNA) production which carries the message of DNA from the nucleus to the cytoplasm. Transfer RNA (tRNA) carries the amino acids to ribosomal RNA (rRNA) to produce polypeptides according to the message on mRNA.