FUTURE OF TISSUE ENGINEERING

Tissue Engineering and Regenerative Medicine International Society is an international learned society. Its goal is the worldwide advancement of both the science and technology of tissue engineering and regenerative medicine.

FUTURE OF TISSUE ENGINEERING :

Patients suffering from diseased and injured organs are often treated with transplanted organs, and this treatment has been in use for over 50 years. In 1955, the kidney became the first entire organ to be replaced in a human, when Murray transplanted this organ between identical twins. Several years later, Murray performed an allogeneic kidney transplant from a non-genetically identical patient into another. This transplant, which overcame the immunologic barrier, marked a new era in medicine and opened the door for use of transplantation as a means of therapy for different organ systems.

Modern medicine increases the human lifespan, the aging population grows, and the need for donor organs grows with it, because aging organs are generally more prone to failure. However, there is now a critical shortage of donor organs, and many patients in need of organs will die while waiting for transplants. In addition, even if an organ becomes available, rejection of organs is still a major problem in transplant patients despite improvements in the methods used for immunosuppression following the transplant procedure. Even if rejection does not occur, the need for lifelong use of immunosuppressive medications leads to a number of complications in these patients.

These problems have led physicians and scientists to look to new fields for alternatives to organ transplantation. In the 1960s, a natural evolution occurred in which researchers began to combine new devices and materials sciences with cell biology, and a new field that is now termed *tissue engineering* was born. As more scientists from different fields came together with the common goal of tissue replacement, the field of tissue engineering became more formally established. Tissue engineering is now defined as "an interdisciplinary field which applies the principles of engineering and life sciences towards the development of biological substitutes that

aim to maintain, restore or improve tissue function."Then, after the discovery of human stem cells by Thomson's group in the early 1980s,the field of stem cell biology took shape and suggested that it may one day be possible to obtain and use donor stem cells in tissue engineering strategies, or perhaps even reactivate endogenous stem cells and use them to regenerate failing organs in adult patients.

The fields of stem cells, cell transplantation, and tissue engineering all have one unifying concept-the regeneration of living tissues and organs. Thus, in 1999, William Haseltine, then the Scientific Founder and Chief Executive Officer of Human Genome Sciences, coined the term *regenerative medicine*.

THE BASIC COMPONENTS OF REGENERATIVE MEDICINE STRATEGIES

The field of regenerative medicine encompasses various areas of technology, such as tissue engineering, stem cells, and cloning. Tissue engineering, one of the major areas of regenerative medicine, follows the principles of cell transplantation, materials science, and engineering toward the development of biological substitutes that can restore and maintain normal function. Tissue engineering strategies generally fall into two categories: the use of acellular scaffolds, which depend on the body's natural ability to regenerate for proper orientation and direction of new tissue growth, and the use of scaffolds seeded with cells. Acellular scaffolds are usually prepared by manufacturing artificial scaffolds or by removing cellular components from tissues via mechanical and chemical manipulation to produce acellular, collagen-rich matrices. These matrices tend to slowly degrade on implantation and are generally replaced by the extracellular matrix (ECM) proteins that are secreted by the in-growing cells. Cells can also be used for therapy via injection, either with carriers such as hydrogels or alone.

1. Biomaterials for use in regenerative medicine

In the past, synthetic materials were introduced to replace or to rebuild diseased tissues or parts in the human body. The manufacture of new materials, such as tetrafluoroethylene (Teflon) and silicone, opened a new field of research that led to the development of a wide array of devices that could be applied for human use. Although these devices could provide structural support or replacement, the functional component of the original tissue was not restored. However, studies in cell biology, molecular biology, and biochemistry allowed a better

understanding of the ECM and its interaction with cells in the tissues of the body, as well as interactions with growth factors and their ligands, and as a result, new biomaterials were designed with these interactions in mind.

In tissue engineering, biomaterials replicate the biological and mechanical function of the native ECM found in tissues in the body. Biomaterials provide a three-dimensional space in which cells can attach, grow, and form new tissues with appropriate structure and function. They also allow for the delivery of cells and appropriate bioactive factors (e.g., cell adhesion peptides, growth factors) to desired sites in the body. Because most mammalian cell types are anchorage-dependent and will die if no cell-adhesion substrate is available, biomaterials provide this substrate while allowing delivery of cells with high loading efficiency. Biomaterials can also provide mechanical support against *in vivo* forces so that the predefined three-dimensional structure of a tissue-engineered organ is maintained during tissue development.

The ideal biomaterial should be biodegradable and bioresorbable to support the replacement of normal tissue without inducing inflammation. Incompatible materials are destined for an inflammatory or foreign-body response that eventually leads to rejection or necrosis. Because biomaterials provide temporary mechanical support while the cells undergo spatial reorganization into tissue, a properly chosen biomaterial should allow the engineered tissue to maintain sufficient mechanical integrity to support itself in early development, while in late development, it should have begun degradation such that it does not hinder further tissue growth.The degradation products, if produced, should be removed from the body via metabolic pathways at an adequate rate to ensure that the concentration of these degradation products in the tissues remains at a tolerable level.

Generally, three classes of biomaterials have been utilized for engineering tissues: naturally derived materials (e.g., collagen and alginate), acellular tissue matrices (e.g., bladder submucosa and small intestinal submucosa),and synthetic polymers such as polyglycolic acid (PGA), polylactic acid (PLA), and poly (lactic-co-glycolic acid) (PLGA).These classes of biomaterials have been tested with respect to their biocompatibility.Naturally derived materials and acellular tissue matrices have the potential advantage of biological recognition. However, synthetic polymers can be produced reproducibly on a large scale with controlled properties such as strength, degradation rate, and microstructure.

2. Cells for use in cell therapy and tissue engineering

1) Native cells

When native cells are used for tissue engineering, a small piece of donor tissue is dissociated into individual cells. These cells are expanded in culture and either injected directly back into the host or attached to a support matrix and then reimplanted. The source of donor tissue can be heterologous (such as bovine), allogeneic (same species, different individual), or autologous. The preferred cells to use are autologous cells, where a biopsy of tissue is obtained from the host, the cells are dissociated and expanded in culture, and the expanded cells are implanted into the same host.The use of autologous cells, although it may cause an inflammatory response, avoids rejection, and thus the deleterious side effects of immunosuppressive medications can be avoided.

Ideally, both structural and functional tissue replacement will occur with minimal complications when autologous native cells are used. However, one of the limitations of applying cell-based regenerative medicine techniques to organ replacement has been the inherent difficulty of growing specific cell types in large quantities. Even when some organs, such as the liver, have a high regenerative capacity *in vivo*, cell growth and expansion *in vitro* may be difficult. By studying the privileged sites for committed precursor cells in specific organs, as well as exploring the conditions that promote differentiation, one may be able to overcome the obstacles that limit cell expansion *in vitro*. For example, urothelial cells could be grown in the laboratory setting in the past, but only with limited expansion. Several protocols were developed over the past two decades that identified the undifferentiated cells and kept them undifferentiated during their growth phase. With the use of these methods of cell culture, it is now possible to expand a urothelial strain from a single specimen that initially covered a surface area of 1 cm^2 to one covering a surface area of 4,202 m^2 (the equivalent of one football field) within 8 weeks. These studies indicated that it should be possible to collect autologous bladder cells from human patients, expand them in culture, and return them to the donor in sufficient quantities for reconstructive purposes. Major advances have been achieved within the past decade on the possible expansion of a variety of primary human cells, with specific techniques that make the use of autologous cells for clinical application possible.

Most current strategies for tissue engineering depend on a sample of autologous cells from the diseased organ of the host. However, for many patients with extensive end-stage organ failure, a tissue biopsy may not yield enough normal cells for expansion and transplantation. In other instances, primary autologous human cells cannot be expanded from a particular organ, such as the pancreas. In these situations, stem cells are envisioned as being an alternative source of cells from which the desired tissue can be derived. Stem cells can be derived from discarded human embryos (human embryonic stem cells), from fetal tissue, or from adult sources (bone marrow, fat, skin).

3. Stem cells for use in tissue engineering

1) Embryonic stem cells

Human embryonic stem (hES) cells exhibit two remarkable properties: the ability to proliferate in an undifferentiated but pluripotent state (self-renewal), and the ability to differentiate into many specialized cell types. They can be isolated by aspirating the inner cell mass from the embryo during the blastocyst stage (5 days post-fertilization) and are usually grown on feeder layers consisting of mouse embryonic fibroblasts or human feeder cells. More recent reports have shown that these cells can be grown without the use of a feeder layer and thus avoid the exposure of these human cells to mouse viruses and proteins. These cells have demonstrated longevity in culture by maintaining their undifferentiated state for at least 80 passages when grown by use of current published protocols. In addition, hES cells are able to differentiate into cells from all three embryonic germ layers *in vitro*. Skin and neurons have been formed, indicating ectodermal differentiation. Blood, cardiac cells, cartilage, endothelial cells, and muscle have been formed, indicating mesodermal differentiation. Pancreatic cells have been formed, indicating endodermal differentiation. In addition, as further evidence of their pluripotency, embryonic stem cells can form embryoid bodies, which are cell aggregations that contain all three embryonic germ layers while in culture and can form teratomas *in vivo*. However, there are many ethical and religious concerns associated with hES cells because embryos are destroyed in order to obtain them. Thus, the use of these cells is currently banned in many countries.

2) Stem cells from somatic cell nuclear transfer

Stem cells for tissue engineering could also be generated through cloning procedures. There has been tremendous interest in the field of nuclear cloning since the birth of the cloned sheep Dolly in 1997, but actually, Dolly was not the first animal produced by using nuclear transfer. In fact, frogs were the first successfully cloned vertebrates derived from nuclear transfer. However, in the frog experiment, the nuclei used for cloning were derived from non-adult sources. In fact, live lambs were produced in 1996 by using nuclear transfer as well, but they were produced from differentiated epithelial cells derived from embryonic discs. The significance of Dolly was that she was the first mammal to be derived from an adult somatic cell by use of nuclear transfer. Since then, animals from several species have been grown by using nuclear transfer technology, including cattle, goats, mice, and pigs.

Two types of nuclear cloning, reproductive cloning and therapeutic cloning, have been described, and a better understanding of the differences between the two types may help to alleviate some of the controversy that surrounds these technologies. Banned in most countries for human applications, reproductive cloning is used to generate an embryo that has the identical genetic material as its cell source. This embryo can then be implanted into the uterus of a female to give rise to an infant that is a clone of the donor. On the other hand, therapeutic cloning is used to generate early stage embryos that are explanted in culture to produce embryonic stem cell lines whose genetic material is identical to that of its source. These autologous stem cells have the potential to become almost any type of cell in the adult body, and thus would be useful in tissue and organ replacement applications. Therefore, therapeutic cloning, which has also been called somatic cell nuclear transfer, may provide an alternative source of transplantable cells. According to data from the Centers for Disease Control and Prevention, an estimated 3,000 Americans die every day of diseases that could have been treated with stem cell-derived tissues.With current allogeneic tissue transplantation protocols, rejection is a frequent complication because of immunologic incompatibility, and immunosuppressive drugs are usually required. The use of transplantable tissue and organs derived from therapeutic cloning could lead to the avoidance of immune responses that typically are associated with transplantation of non-autologous tissues.

While promising, somatic cell nuclear transfer technology has certain limitations that require further study before this technique can be applied widely in tissue or organ replacement therapy. First, the efficiency of the cloning process is very low, as evidenced by the fact that most embryos derived from the cloning process do not survive. To improve cloning efficiency, further improvements are required in many of the complex steps of nuclear transfer, such as the enucleation process for oocytes, the actual transfer of a nucleus to this enucleated oocyte, and the activation process that instructs the cloned oocytes to begin dividing. In addition, cell cycle synchronization between donor cells and recipient oocytes must be accomplished.

3) Reprogramming and generation of iPS cells

Within the past few years, exciting reports of the successful transformation of adult somatic cells into pluripotent stem cells through genetic "reprogramming" have been published. Reprogramming is a technique that involves de-differentiation of adult somatic cells (such as fibroblasts) to produce patient-specific pluripotent stem cells. This process is especially exciting because it allows pluripotent stem cells to be obtained without the use of embryos. Also, cells generated by reprogramming are genetically identical to the somatic cells used (and thus to the patient who donated these cells) and should not be rejected. Yamanaka was the first to discover that mouse embryonic fibroblasts (MEFs) and adult mouse fibroblasts could be reprogrammed into an "induced pluripotent state (iPS)." They examined 24 genes that were thought to be important for embryonic stem cells and identified 4 key genes that, when introduced into the reporter fibroblasts via retroviral vectors, resulted in drug-resistant cells. These were *Oct3/4*, *Sox2*, *c-Myc*, and *Klf4*. The resultant iPS cells possessed the immortal growth characteristics of self-renewing embryonic stem cells, expressed genes specific for embryonic stem cells, and generated embryoid bodies *in vitro* and teratomas *in vivo*. When iPS cells were injected into mouse blastocysts, they contributed to a variety of cell types. However, although iPS cells selected in this way were pluripotent, they were not identical to embryonic stem cells. Unlike embryonic stem cells, chimeras made from iPS cells did not result in full-term pregnancies. Gene expression profiles of the iPS cells showed that they possessed a distinct gene expression signature that was different from that of embryonic stem cells. In addition, the epigenetic state of the iPS cells was somewhere between that found in somatic cells and that found in embryonic stem cells, suggesting that the reprogramming was incomplete.

These results were improved significantly by Wernig and Jaenisch in July 2007. Fibroblasts were infected with retroviral vectors and selected for the activation of endogenous *Oct4* or *Nanog* genes. Results from this study showed that DNA methylation, gene expression profiles, and the chromatin state of the reprogrammed cells were similar to those of embryonic stem cells. Teratomas induced by these cells contained differentiated cell types representing all three embryonic germ layers. Most importantly, the reprogrammed cells from this experiment could form viable chimeras and contribute to the germline-like embryonic stem cells, suggesting that these iPS cells were completely reprogrammed. Wernig et al observed that the number of reprogrammed colonies increased when drug selection was initiated later (day 20 rather than day 3 post-transduction). This suggests that reprogramming is a slow and gradual process and may explain why previous attempts resulted in incomplete reprogramming.

It has recently been shown that reprogramming of human cells is possible. Yamanaka generated human iPS cells that are similar to hES cells in terms of morphology, proliferation, gene expression, surface markers, and teratoma formation. Thompson's group showed that retroviral transduction of the stem cell markers *OCT4*, *SOX2*,*NANOG*, and *LIN28* could generate pluripotent stem cells. However, in both studies, the human iPS cells were similar but not identical to hES cells. Although reprogramming is an exciting phenomenon, our limited understanding of the mechanism underlying it currently limits the clinical applicability of the technique, but the future potential of reprogramming is quite exciting.

4) Amniotic fluid and placental stem cells

An alternate source of stem cells is the amniotic fluid and placenta. Amniotic fluid and the placenta are known to contain multiple partially differentiated cell types derived from the developing fetus. We isolated stem cell populations from these sources, called amniotic fluid and placental stem cells (AFPSC), that express embryonic and adult stem cell markers. The undifferentiated stem cells expand extensively without feeders and double every 36 hours. Unlike hES cells, the AFPSC do not form tumors *in vivo*. Lines maintained for over 250 population doublings retained long telomeres and a normal karyotype. AFS cells are broadly multipotent. Clonal human lines verified by retroviral marking can be induced to differentiate into cell types representing each embryonic germ layer, including cells of adipogenic, osteogenic, myogenic, endothelial, neuronal, and hepatic lineages. In this respect, they meet a commonly accepted criterion for pluripotent stem cells, without implying that they can generate every adult tissue. Examples of differentiated cells derived from AFS cells and displaying

specialized functions include neuronal lineage cells secreting the neurotransmitter L-glutamate or expressing G-proteingated inwardly rectifying potassium (GIRK) channels, hepatic lineage cells producing urea, and osteogenic lineage cells forming tissue engineered bone. The cells could be obtained either from amniocentesis or chorionic villous sampling in the developing fetus, or from the placenta at the time of birth. The cells could be preserved for self-use and used without rejection, or they could be banked. A bank of 100,000 specimens could potentially supply 99% of the US population with a perfect genetic match for transplantation. Such a bank may be easier to create than with other cell sources, because there are approximately 4.5 million births per year in the USA.

5) Adult stem cells

Adult stem cells, especially hematopoietic stem cells, are the best understood cell type in stem cell biology. The presence of stem cells in the adult was first discerned by Till and McCulloch, who were investigating the mechanisms by which the bone marrow could regenerate after exposure to radiation. However, adult stem cell research remains an area of intense study, because their potential for therapy may be applicable to a myriad of degenerative disorders. Within the past decade, adult stem cell populations have been found in many adult tissues other than the bone marrow and the gastrointestinal tract, including the brain, skin, and muscle. Many other types of adult stem cells have been identified in organs all over the body and are thought to serve as the primary repair entities for their corresponding organs. The discovery of such tissue-specific progenitors has opened up new avenues for research.

A notable exception to the tissue-specificity of adult stem cells is the mesenchymal stem cell (MSC), also known as the multipotent adult progenitor cell. This cell type is derived from bone marrow stroma. Such cells can differentiate *in vitro* into numerous tissue types and can also differentiate developmentally if injected into a blastocyst. Multipotent adult progenitor cells can develop into a variety of tissues including neuronal, adipose, muscle, liver, -lungs, spleen, and gut tissue but notably not bone marrow or gonads.

In addition, stem cells derived from adipose tissue may also be an autologous and self-renewing cell source. Adipose-derived stem cells (ADSCs) have been shown to differentiate into a variety of cell phenotypes, and since they are easily obtained, they show great promise for future types of reconstructive surgery based on tissue engineering and there have been several clinical trials using these cells. Wilson and Mizuno have both provided excellent, detailed reviews of these.

Research into more differentiated types of adult stem cells has, however, progressed slowly, mainly because investigators have had great difficulty in maintaining adult non-mesenchymal stem cells in culture. Some cells, such as those of the liver, pancreas, and nerve, have very low proliferative capacity *in vitro*, and the functionality of some cell types is reduced after the cells are cultivated. Isolation of cells has also been problematic, because stem cells are present in extremely low numbers in adult tissue. While the clinical utility of adult stem cells is currently limited, great potential exists for future use of such cells in tissue-specific regenerative therapies. The advantage of adult stem cells is that they can be used in autologous therapies, thus avoiding any complications associated with immune rejection.

TRANSGENESIS- METHODS OF GENE TRANSFER:

Transgenesis is the process of introducing an exogenous gene — called a transgene — into a living organism so that the organism will exhibit a new property and transmit that property to its offspring. Transgenesis can be facilitated by liposomes, plasmid vectors, viral vectors, pronuclear injection, protoplast fusion, and ballistic DNA injection.

Transgenic organisms are able to express foreign genes because the genetic code is similar for all organisms. This means that a specific DNA sequence will code for the same protein in all organisms. Due to this similarity in protein sequence, scientists can cut DNA at these common protein points and add other genes. An example of this is the "super mice" of the 1980s. These mice were able to produce the human protein tPA to treat blood clots.

Transgenic Animals: Objectives of Gene Transfer with Different Transfection Methods!

A transgenic animal contains in its genome, a gene or genes introduced by one or the other technique of transfection.

The gene introduced by transfection is called a transgene. In animals transfection specifies the introduction of a DNA segment, either naked or integrated into a vector, into an animal cell.

Objectives of Gene Transfer:

1. Genetic modification of animals may be aimed at improving their milk, meat, wool, etc. production.

2. Genes have been transferred into animals to obtain a large scale production of the proteins encoded by these genes in the milk, urine or blood of such animals.

3. A special case of gene transfer aims at alleviating or even eliminating the symptoms and consequent miseries of genetic diseases. In this approach, normal and functional copies of the defective gene are introduced into the patient (gene therapy).

4. Specific transgenic animal strains or lines are created to fulfill specialized experimental and/or biomedical needs.

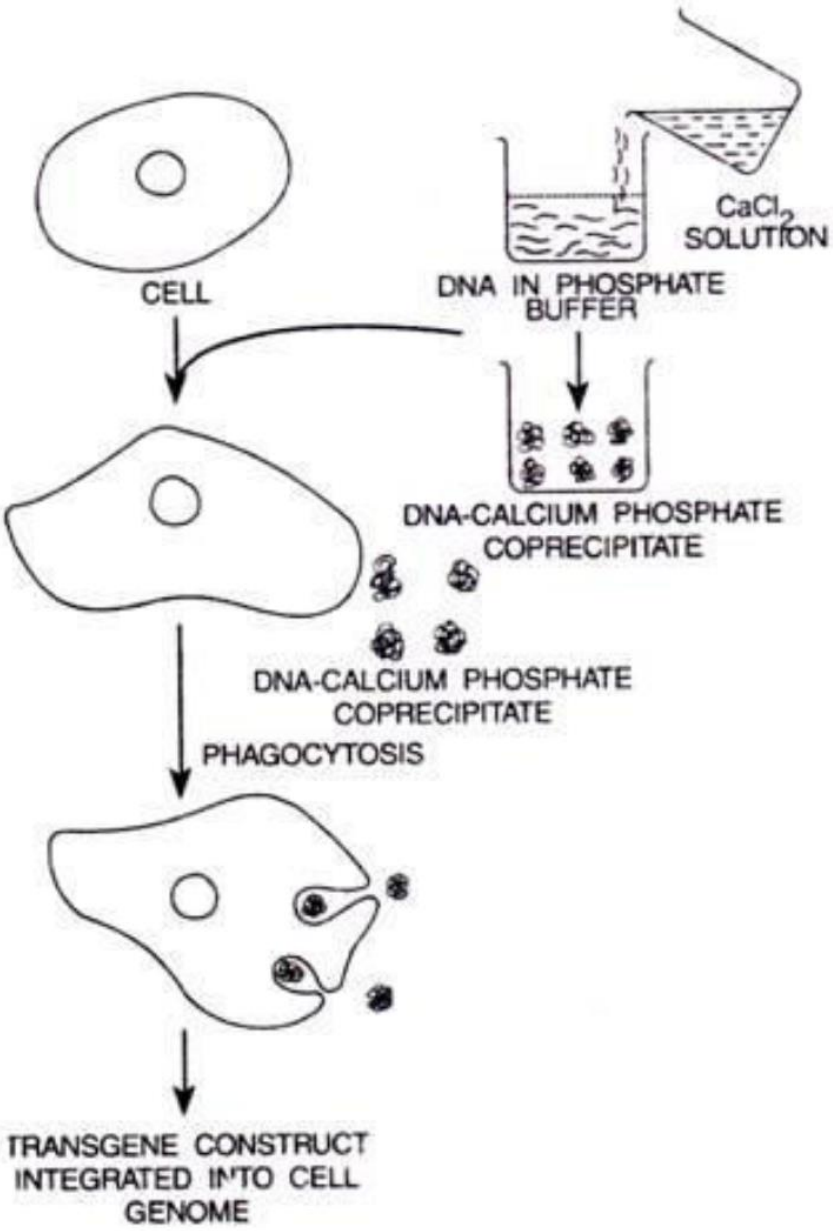
Transfection Methods:

Calcium Phosphate Precipitation:

In this approach, the DNA preparation to be used for transfection is first dissolved in a phosphate buffer. Calcium chloride solution is then added to the DNA solution; this leads to the formation of insoluble calcium phosphate which co-precipitates with the DNA. The calcium phosphate-DNA precipitate is added to the cells to be transfected.

The precipitate particles are taken in by the cells by phagocytosis. Initially, 1-2% of the cells were transfected by this approach. But the procedure has now been modified to obtain transfection of upto 20% of the cells. In a small proportion of the transfected cells, the DNA becomes integrated into the cell genome producing stable or permanent transfection.

This general approach can be applied to virtually all mammalian cells, and a very large number of cells can be treated with little effort. But many cell lines do not like the calcium phosphate precipitate adhering to their surfaces or to their substrate.



DEAE-Dextran-Mediated Transfection:

DEAE-dextran (dimethylaminoethyl-dextran) is water soluble and polycationic, i.e., has a multiple positive charge. It is added to the transfection solution containing the DNA. In some unknown way, DEAE-dextran brings about DNA uptake by the cells through endocytosis.

Possibly its interaction with the negatively charged DNA molecules and with the components of cell surface plays an important role. This procedure is highly suited for transient transfection used for various molecular biology studies, particularly using COS cell lines. However, again for some unknown reason, it is not efficient in producing stable transfection.

Lipofection:

The delivery of DNA into cells using liposomes is called lipofection. Liposomes are small vesicles prepared from a suitable lipid. Initially, nonionic lipids were used for preparing liposomes so that DNA had to be introduced within the vesicles following specific encapsidation procedures.

The use of cationic lipids for the construction of liposomes is a distinct advantages as DNA spontaneously and efficiently complexes with these liposomes making encapsidation procedures unnecessary. The cationic liposomes have a single lipid bilayer membrane (unilamellar), and they bind to the cells efficiently. Probably they fuse with the plasma membrane and thereby deliver the DNA (complexed with them) into the cells, which brings about transfection.

Lipofection is the method of choice for transfection of mammalian cells in vitro. It has also been used to deliver DNA into live animals by direct injection or intravenous injection. Cationic liposomes have been used in intravenous or intratracheal injection in mice for the expression of marker genes in lungs. Targeted delivery has also been demonstrated by incorporating specific ligand proteins into the liposome membranes.

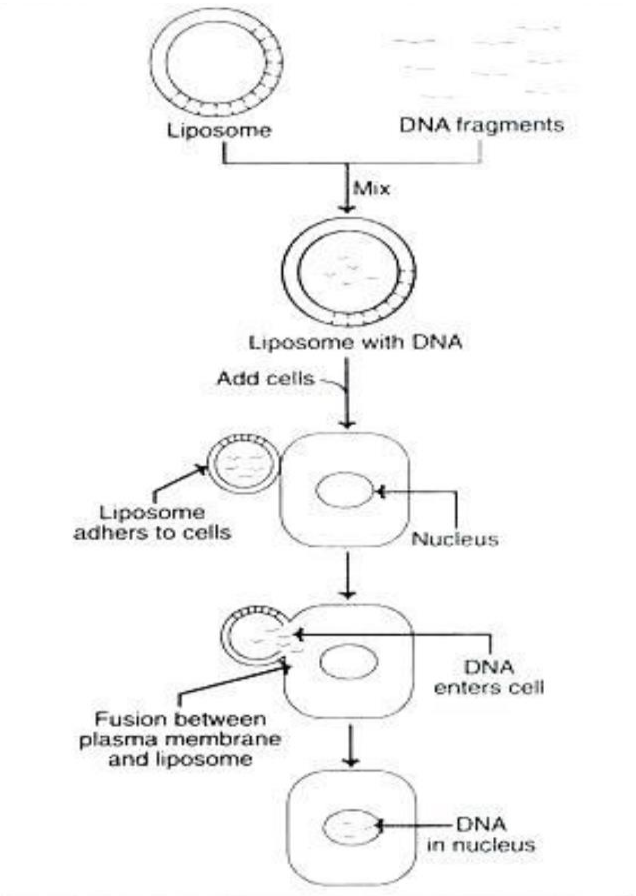
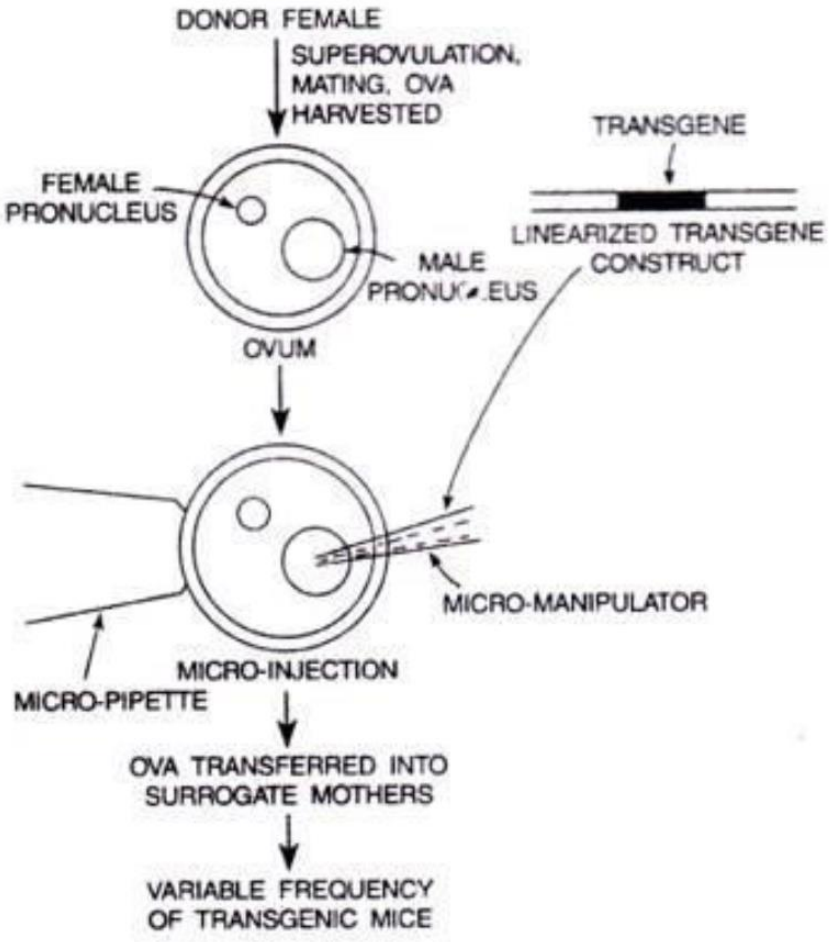


Fig. 6.12 : Liposome-mediated gene transfer
(Note : For clarity, the native cell DNA is not shown).

Microinjection:

In this method, DNA solution is injected directly into the nucleus of a cell or into the male pronucleus of a fertilized one to two-cell ovum. Typically, a microinjection assembly consists of a low power stereoscopic dissecting microscope (to view the ovum and the entire process) and two micromanipulators, one for a glass micropipette to hold the ovum by partial suction and the other for a glass injection needle to introduce the DNA into the male pronucleus. The male pronucleus is much larger than the female pronucleus of fertilized mammalian ova. However in fish ova, the DNA is injected into the egg cytoplasm.

The general procedure for microinjection is as follows: Donor females are induced to super-ovulate using appropriate hormone treatments. The superovulated females are then mated with fertile males, and large numbers of fertilized one or two-cell ova/embryos are collected surgically. Alternatively, unfertilized ova are collected from super-ovulated females; the ova are then fertilized in vitro. The transgene construct is prepared in a buffer solution and is injected into the male pronuctei of fertilized eggs using a microinjection assembly.



PARTICLE GUN/BIOLISTICS:

A gene gun or a biolistic particle delivery system, originally designed for plant transformation, is a device for injecting cells with genetic information; the inserted genetic material are termed transgenes. The payload is an elemental particle of aheavy metal coated with plasmid DNA. This technique is often simply referred to as bioballistics or biolistics.

This device is able to transform almost any type of cell, including plants, and is not limited to genetic material of the nucleus: it can also transform organelles, including plastids.

Biolistic construct design:

A construct is a piece of DNA inserted into the target's genome, including parts that are intended to be removed later. All biolistic transformations require a construct to proceed and while there is great variation among biolistic constructs, they can be broadly sorted into two categories: those which are designed to transform eukaryotic nuclei, and those designed to transform prokaryotic-type genomes such as mitochondria, plasmids or plastids.

Those meant to transform prokaryotic genomes generally have the gene or genes of interest, at least one promoter and terminator sequence, and a reporter gene; which is a gene used to ease detection or removal of those cells which didn't integrate the construct into their DNA. These genes may each have their own promoter and terminator, or be grouped to produce multiple gene products from one transcript, in which case binding sites for translational machinery should be placed between each to ensure maximum translational efficiency. In any case the entire construct is flanked by regions called border sequences which are similar in sequence to locations within the genome, this allows the construct to target itself to a specific point in the existing genome.

Constructs meant for integration into a eukaryotic nucleus follow a similar pattern except that: the construct contains no border sequences because the sequence rearrangement that prokaryotic constructs rely on rarely occurs in eukaryotes; and each gene contained within the construct must be expressed by its own copy of a promoter and terminator sequence.

Though the above designs are generally followed, there are exceptions. For example, the construct might include a Cre-Lox system to selectively remove inserted genes; or a prokaryotic construct may insert itself downstream of a promoter, allowing the inserted genes to be governed by a promoter already in place and eliminating the need for one to be included in the construct.

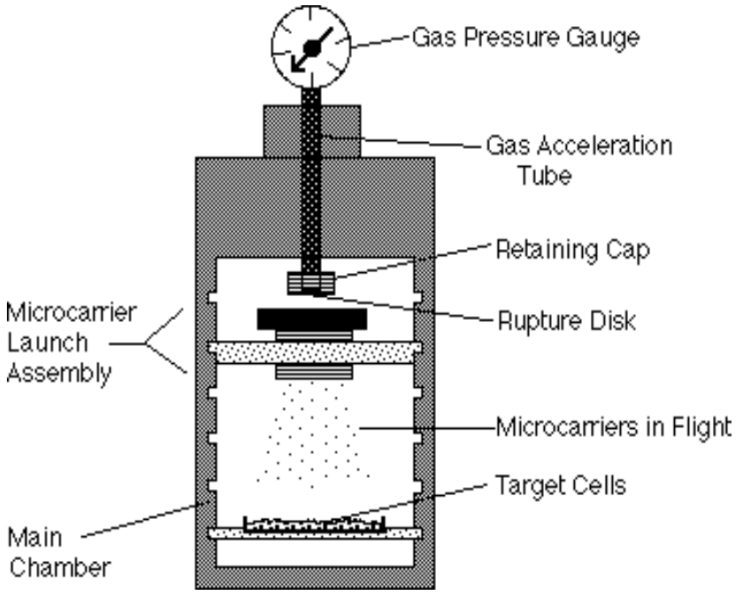
APPLICATIONS:

Gene guns have also been used to deliver DNA vaccines.

The delivery of plasmids into rat neurons through the use of a gene gun, specifically DRG neurons, is also used as a pharmacological precursor in studying the effects of neurodegenerative diseases such as Alzheimer's disease.

The gene gun has become a common tool for labeling subsets of cells in cultured tissue. In addition to being able to transfect cells with DNA plasmids coding for fluorescent proteins, the gene gun can be adapted to deliver a wide variety of vital dyes to cells.

Gene gun bombardment has also been used to transform *Caenorhabditis elegans*, as an alternative to microinjection.



Electroporation:

In this approach, transfection mixture containing cells and DNA is exposed for a very brief period (few milliseconds) to a very high voltage gradient (e.g., 4,000-8,000 V/cm). Electroporation is generally done at room temperature, but the cells are subsequently kept on ice to allow the membrane pores to remain open for a longer duration.

This induces transient pores in the cell membranes through which DNA seems to enter the cells. Treatment of cells with colcemid before they are electroporated increases the frequency of

transfection. This is most likely due to the arrest of cells at metaphase and the associated absence of nuclear envelope or to an unusual permeability of the plasma membranes.

Linearized DNA is far more efficient in transfection than circular supercoiled DNA possibly because of a higher frequency of integration of linear DNA into the genome. Generally, DNA integration occurs in a low copy number, while chemical methods usually lead to multiple copy integration per genome. Electroporation technique has a general applicability, and many animal cell types that could not be transfected by other approaches were successfully transfected by this approach.

A single cell electroporation microarray based on a silicon chip of 1 cm^2 has been developed. The chip has 60 circular, cell-sized (20 pm diameter) microelectrodes each one of which is contacted by a dedicated neutral line. The silicon chip is placed in a plastic chamber designed to hold culture medium. The cells are cultured on the chip surface, and they can be individually electroporated by a PC-driven control system. The DNA can be delivered to several preselected individual cells within the same culture, at arbitrarily chosen time points, and even sequentially to the same cell.

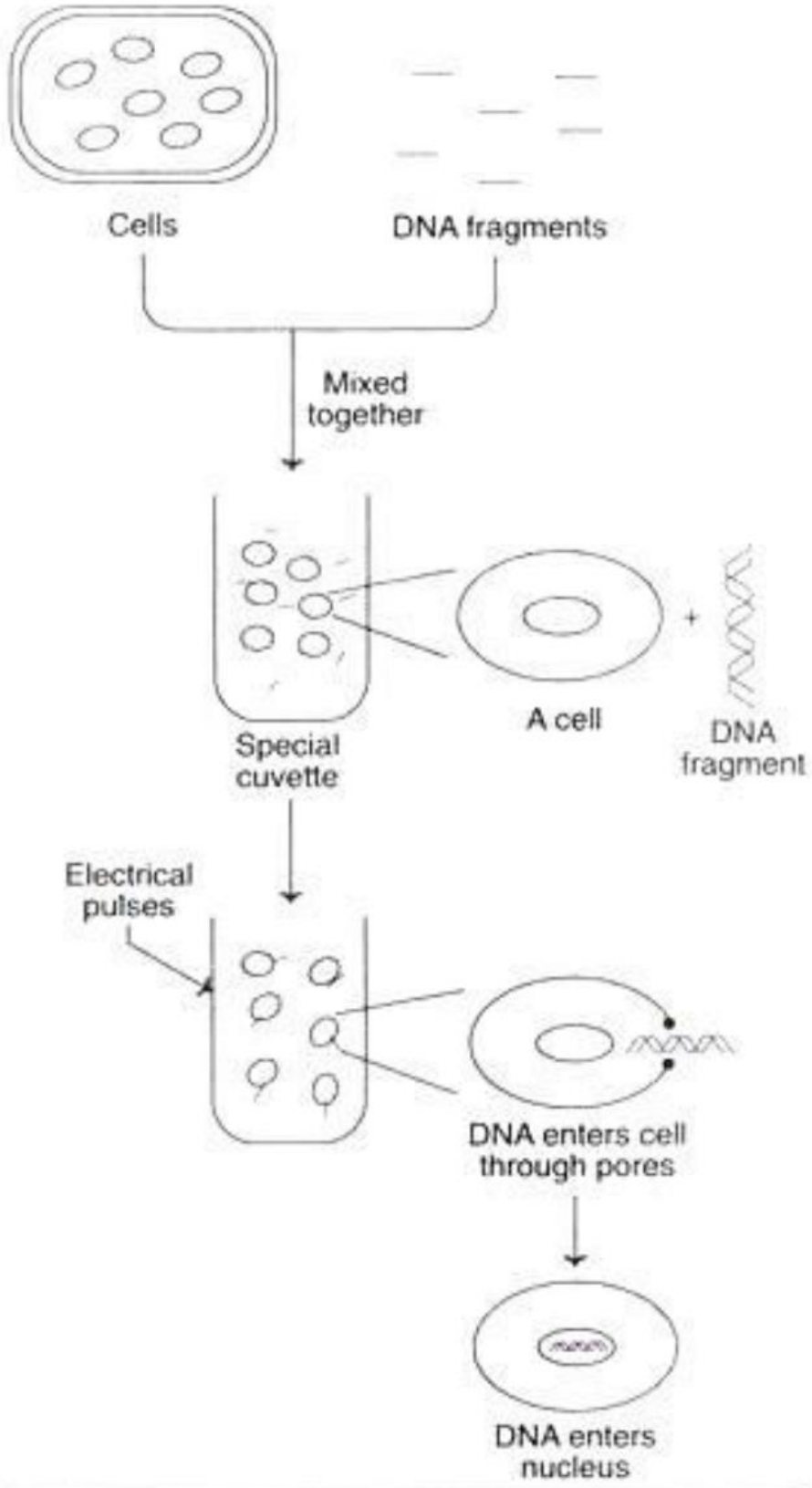


***Fig. 6.11** : Gene transfer by electroporation.*
***(Note** : Magnification depicted on right side)*

References and further reading

Tissue Engineering--Current Challenges and Expanding Opportunities by Linda G. Griffith and Gail Naughton (2002)

Textbook of Animal Biotechnology, by B Singh (Author), S K Gautam (Author), M S Chauhan (Author)

Animal Biotechnology (3rd Edition), by M.M. Ranga (Author)