***Module objective***

*This module attempts to introduce the learner to different strategies involved in tissue regeneration. Various factors influencing the efficiency of regeneration are dealt with in detail*

## Preface

**"***If we can reduce the cost and improve the quality of medical technology through advances in nanotechnology, we can more widely address the medical conditions that are prevalent and reduce the level of human suffering*"said the eminent scientist and molecular nanotechnologist Ralph Merkle. The advent of nanotechnology and the discovery of the complex networks and stimuli that regulate the cell-fate processes have resulted in rapid advances in the healthcare scenario. An exciting field that is the offspring of the alliance between nanotechnology and cell biology has been tissue engineering. The following set of four lectures will highlight the influence of various parameters on achieving tissue regeneration.

*This lecture deals with the description of the first major component of the tissue engineering triad – the cells.*

## 1 What is tissue engineering?

The concept of tissue engineering involves the integration of multiple components to bring about organized cell growth to replace diseased tissue without compromising on the biological functions of the cells. In order to regenerate a tissue, the cells must exhibit a regulated as well as oriented growth. They must be securely adhered on to a substrate and must not be floating about. They also must exhibit the characteristic phenotype of the cell as well as the functions specific to the cell type. Both cell-cell contacts as well as cell-substrate contacts must be well established. In order to achieve these characteristics, the environment in which the cells are grown must be carefully regulated. Let us see how this can be achieved in the following discussion.

## 2 Components essential for tissue engineering

There are three main components involved in the tissue regeneration process that is referred to as the *'tissue engineering triad'*. These components are the **cells** used to regenerate the tissue, the **substrate**or**scaffold** on which these cells grow and finally the **chemical molecules** that are responsible to stimulate the cell functions. Each component has several parameters that can influence the cell growth. The figure 1 gives a schematic representation of the key factors that influence the tissue engineering components.

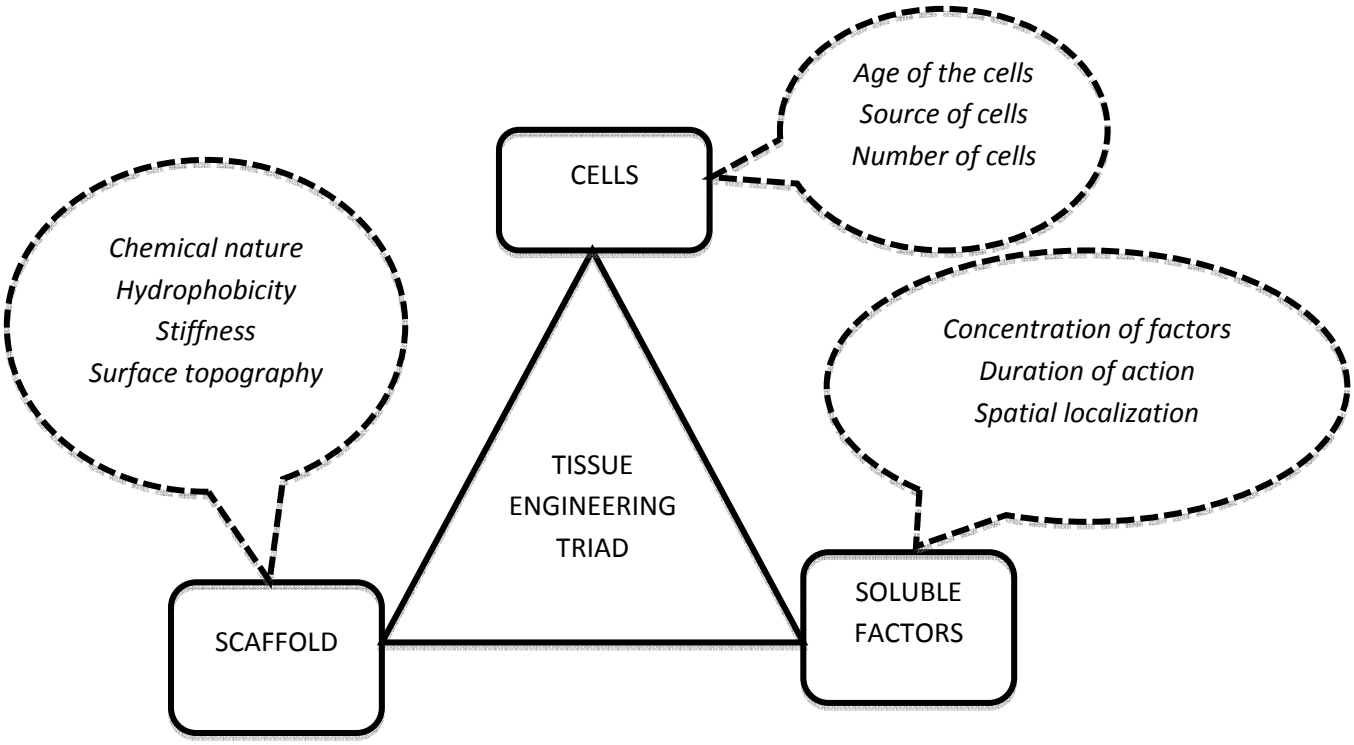


**Fig. 1: Schematic representation of factors that influence tissue engineering**

## 3 Cells

Let us consider the first and a vital component of the tissue engineering triad – Cells!One of the important factors in tissue engineering is to ensure that the cells adhere, grow, proliferate and function in the substrate provided so that the three-dimensional tissue architecture is achieved. The number of cells that is added to the substrate (a process referred to as seeding) will determine the speed with which the cells cover the substrate. Very small cell numbers will result in extremely poor rates of surface coverage as the cells must divide, grow and then try to establish contact with its neighbouring cell. On the other hand, very high cell numbers also are not favourable for tissue engineering as there will be a lot of competition between the cells for space as well as nutrients. This will result in cell death! Thus an optimum number of cells should be seeded on the substrate. But this optimum number will vary depending on the cell type. In other words, the initial number of cells seeded on the substrate will be dependent on the doubling time or proliferating rate of the cells. For example, chondrocytes are very slow to proliferate and hence the seeding density (the number of cells seeded per unit volume on the substrate) required will be high. On the other hand, fibroblasts have a very fast proliferation rate and hence even small seeding densities can result in complete surface coverage in short duration. Now, you may wonder as to why is it so important to have fast cell coverage of the surface. Simple! If the duration taken for complete surface coverage of the substrate by cells is very long, then the

possibilities of protein adsorption will increase. If the complement proteins adsorb on the substrate, then immune activation occurs. Hence, it is essential that the cells cover the substrate completely.

Well, that explains the importance of cell numbers and also the cell type. The next factor that needs to be addressed in terms of the cells used is the age of the cells. When older cells are employed for regenerating the tissues, then they tend to enter the senescence phase quickly. *(Senescence is a stage when the cell metabolism starts slowing down and they tend to move on quickly to the apoptotic or programmed cell death phase).*As a result, the growth, proliferation and the cell functions are all retarded leading to the failure of the regeneration strategy. Therefore, it is important to use young cells.

The source of the cells also has an important implication on the regeneration potential. The cells can be primary cells, cell lines or stem cells. Primary cells are those that are isolated from the tissue and then cultured. But sufficient number of primary cells may not be available for all tissues and may be difficult to obtain from patients. The age of the cells may also be an issue associated with use of primary cells. Another complication in the use of primary cells is that the isolation procedure might result in a heterogeneous population of cells. These cells need to be sorted to obtain a single lineage of cells or else if used as such, one might not achieve regeneration of the tissue of interest. Use of primary cells from other individuals may lead to immune rejection.

Cell lines are those cells that have been isolated from a source and have been maintained under laboratory conditions over a long period of time. Each time the cells proliferate and reach a certain number, beyond which the cells might start dying. They are then divided into equal numbers and seeded onto another substrate and allowed to proliferate. This is known as ‘passaging the cells’ in cell culture terminology. Such passaging is continuously maintained to keep the cells alive in culture. Most studies employ such cell lines to determine the efficiency of their regeneration strategy. However, a major drawback of using cell lines is that prolonged culture in simulated conditions as maintained during cell culture makes the cells lose their natural functions. Why? It seems that cell in biological system is regulated by other types of cells from other tissues, which is lacking in the culture conditions! Thus, the cell lines do not represent the ideal candidate for tissue regeneration! This leaves one with the option of using stem cells for tissue regeneration applications.

# 4 What are stem cells?

*Did you know?....*

Why are stem cells called so? It is thought that the word 'stem cell' is derived from the German term *stammzelle*, which refers to an ancestor single cell that gives rise to a multicellular organism. This term was first used by the German biologist Ernst Haeckel in 1868!

Stem cells are those cells that can differentiate into any cell type under favourable conditions. In other words, a fully differentiated cell has a pre-defined set of functions that it can perform i.e., it is 'committed'! A stem cell has no specific set of functions assigned to it, but has an ability to 'commit' itself to a particular cell type under suitable conditions. Why are stem cells present in the body? These are believed to be reservoirs of cells that can be called upon in case of any damage to existing cells. In common parlance, these can be called as 'reserve cells'. The ability of the stem cell to differentiate into different cell types is referred to as its '*potency*'.Stem cells have a unique property by which they can renew themselves (self-renewal) in the same undifferentiated form and still retain the ability to differentiate into different cell types. This characteristic is known as '*stemness*'.

# 5 Types of stem cells

Based on its potency, stem cells are classified as:

- Totipotent or Omnipotent
- Pluripotent
- Multipotent
- Oligopotent

A totipotent cell has the ability to differentiate into any cell lineage and hence have the ability to form an entire organism! The early embryonic stem cells or the fertilized egg comes under this category. However, use of a fertilized egg for therapeutic purpose is banned due to ethical concerns.Pluripotent cells are derived from totipotent cells and can differentiate into any cell lineage derived from the three germ layers (endoderm, mesoderm and ectoderm). The later stage embryonic stem cells come under this category.As they are also derived from the embryo, the use of these cells is banned for ethical reasons. The multipotent stem cells have limitations in the number of cells it can differentiate into. Generally, multipotent stem cells tend to differentiate only to related cell lineages based on its origin. For example, hematopoietic stem cells are blood stem cells and have the ability to differentiate into lymphocytes, monocytes, macrophages, erythrocytes, neutrophils, eosinophils etc. Similarly, mesenchymal stem cells are stromal stem cells that can differentiate into osteocytes (bone), chondrocytes (cartilage) and adipocytes (fat cells). A sub-category of multipotentstem cells is the oligopotent stem cells that can differentiate

to even lesser number of cell types. For example, myeloid stem cells or lymphatic stem cells can differentiate into B and T lymphocytes but not into any other type of blood cell. Figure 2depicts the different types of stem cells classified based on their potency.

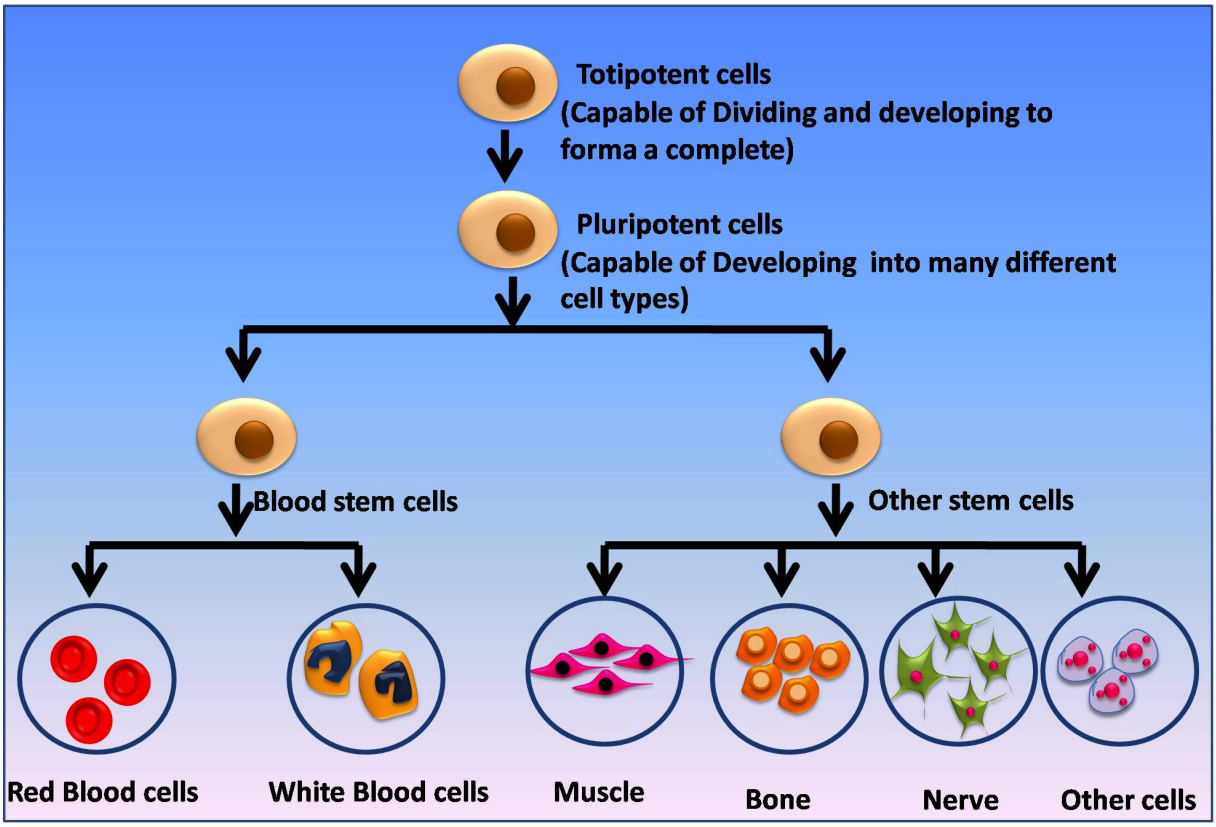


**Fig. 2: Different types of stem cells**

## *5.1 Embryonic and adult stem cells*

Another type of classification of stem cells is based on their origin. Two major categories in this form of classification are the embryonic stem cells and adult or somatic stem cells. While the embryonic stem cells are derived from the embryo, adult stem cells are undifferentiated cells found in developed tissues. They have the ability to self-renew and can differentiate into related cell types and hence are multipotent. Their role in these tissues is to replenish dead cells in these tissues. The adult stem cells do not suffer from ethical issues as they are derived from adult tissues that are generally discarded. However, the number of adult stem cells derived from these tissues is very less. Adult stem cells have been reported from nearly all types of tissues that include adipose, hair follicles, nerves, skin etc.

## *5.2 Induced pluripotent stem cells (iPS)*

Induced pluripotent stem cells were first reported in the year 2006. These stem cells are derived from differentiated somatic cells by 'reprogramming' them to get back into the undifferentiated state. These reprogrammed cells behave almost like pluripotent stem cells and can differentiate into cell lineages derived from the three germ layers. The reprogramming is done by introducing stem cell-specific genes into the cells by virus-mediated transfection. The stem cell-specific genes introduced are Oct-3/4, SOX2, c-Myc, Klf4, Nanog, Glis1 and LIN28. Though this method overcomes the shortcomings of both embryonic and adult stem cells, there are still some unresolved issues that need to be sorted before large scale employment of these iPS cells for tissue engineering. These shortcomings are the poor transformation rate, the risk of developing mutations in the genome as well as induction of cancer. In some cases, incomplete reprogramming may pose a grave danger to the organism. Figure 3 depicts the concept of induced pluripotent cells.

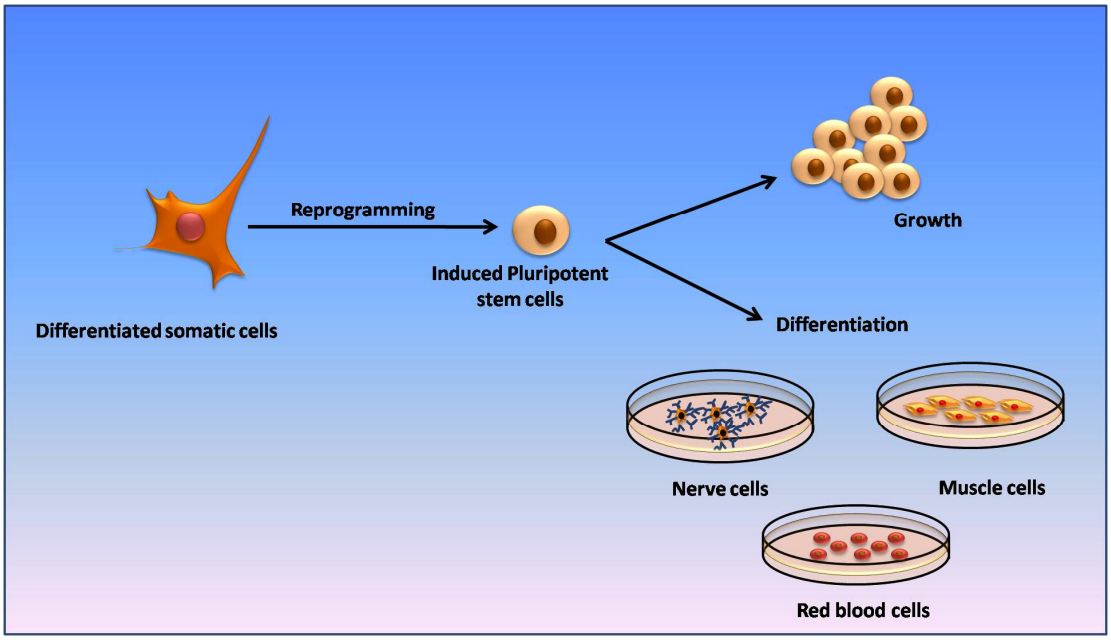


**Fig. 3: Concept of induced pluripotent cells**

*Did you know?....*

- Shinya Yamanaka and John Gurdon received the Nobel prize for medicine in 2012 for their discovery of induced pluripotent stem cells!
- Shinya Yamanaka has been in the forefront in obtaining approval for the world's first clinical trial involving iPS for treatment of age related macular degeneration.

How can one identify a stem cell from a differentiated cell? Well, the stem cell has certain unique surface proteins that distinguish it from other cells. The type of stem cell markers varies depending upon the source of the stem cells. Some of the prominent stem cell markers are: CD9, CD10, CD13, CD29, CD44, CD54, CD105, CD117, CD166, STRO-1 etc.

## 6 Therapeutic applications of stem cells

One of the major questions that arise is, if the stem cells are present even in the adult developed tissues, then why do they lack the ability to replace diseased portions? The reason is that these cells need to be triggered to undergo self-renewal or differentiation. The factors that induce this pathway *in vivo* are yet to be clearly identified. Introduction of stem cells into a diseased region has been found to be beneficial as the tissue-associated factors have been able to direct the differentiation of the stem cells in to the cells of that particular tissue. However, the stem cells also exhibit a tendency to migrate away from the site of introduction. This not only reduces the therapeutic efficiency, but also introduces an additional risk of possible tumor growth at some remote location. Hence, regenerative medicine strategies employing stem cells are exploring the option of seeding scaffolds with stem cells and introduce factors that direct the differentiation of the stem cells in to a desired lineage. This strategy not only retains the stem cells at the site of action but also enhances the regeneration of the lost tissue by bringing about the differentiation of these stem cells. Recently, it has been found that even mechanical stimuli can induce differentiation of stem cells. However, the type of stress, direction, duration, magnitude and frequency of the stress need to be thoroughly investigated.

## 7 Reference

Stem cells: a revolution in therapeutics - Recent advances in stem cell biology and theirtherapeutic applications in regenerativemedicine and cancer therapies, M Mimeault, R Hauke and SK Batra, Clinical Pharmacology & Therapeutics, 82(3), 2007, 252-264

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