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Crossing Horizon In Regeneration

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***ABSTRACT*:** *It is only in the recent past that research on regenerative medicine/dentistry has gained momentum and eluded the dramatic yet scientific advancements in the field of molecular biology. The growing understanding of biological concepts in the regeneration of oral/dental tissues coupled with experiments on stem cells is likely to result in a paradigm shift in the therapeutic armamentarium of dental and oral diseases culminating in an intense search for "biological solutions to biological problems." Stem cells have been successfully isolated from variety of human tissues including orofacial tissues. This review glides us on journey of the origin of stem cells, their properties, characteristics, current research, and their potential applications. It also focuses on the various challenges and barriers that we have to surmount before translating laboratory results to successful clinical applications heralding the dawn of regenerative dentistry.*

KEYWORDS- *Growth factors, pulp regeneration, scaffolds, stem cells, tissue engineering.*

I. INTRODUCTION

Oral tissue regeneration affected by inherited disorders, trauma, and neoplastic or infectious diseases is expected to solve numerous dental problems. In the near future, unparalleled advances in dentistry and endodontics are set to take place, with the availability of artificial teeth, bone, organs, and oral tissues[2,3] as well as the ability to stimulate endodontic regeneration[4]. Patient demand for tissue engineering therapy is staggering both in scope and cost. The endodontic specialty may be able to adopt many of these new scientific advances emerging from regenerative medicine, thereby developing regenerative endodontic procedures and improving patient care.

Regenerative endodontic procedures can be defined as biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex.

II. OBJECTIVES

The objectives of regenerative endodontic procedures are to regenerate pulp-like tissue, ideally, the pulp-dentin complex; regenerate damaged coronal dentin, such as following a carious exposure; and regenerate resorbed root, cervical or apicaldentin.

In the future, the scope of regenerative endodontics may be increased to include the replacement of periapical tissues, periodontal ligaments, gingiva, and even whole teeth.

III. AN OVERVIEW OF REGENERATIVE MEDICINE

Regenerative medicine can perhaps be best defined as the use of a combination of cells, engineering materials, and suitable biochemical factors to improve or replace biological functions in an effort to effect the advancement of medicine. The basis for regenerative medicine is the utilization of tissue engineering therapies. Probably the first definition of tissue engineering was by Langer and Vacanti [5] who stated it was "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function."

MacArthur and Oreffo [6] defined tissue engineering as "understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use."

Our own description goes on to say that "tissue engineering is the employment of biologic therapeutic strategies aimed at the replacement, repair, maintenance, and/or enhancement of tissue function."

The principles of regenerative medicine can be applied to endodontic tissue engineering. Regenerative endodontics comprises research in adult stem cells, growth factors, organ-tissue culture, and tissue engineering materials (Fig .no. 1,2).

IV. ADULT STEM CELLS

All tissues originate from stem cells[7]. A stem cell is commonly defined as a cell that has the ability to continuously divide and produce progeny cells that differentiate (develop) into various other types of cells or tissues.[8]. Stem cells are commonly defined as either embryonic/ fetal or adult/postnatal[9].

Researchers have traditionally found the plasticity of embryonic stem cells to be much greater than that of postnatal stem cells, but recent studies indicate that postnatal stem cells are more plastic than first imagined[10]. Stem cells are also commonly subdivided into totipotent, pluripotent, and multipotent categories according to their plasticity. (Fig.no.3,4,5)

Types of stem cells:

Stem cell type	Cell Plasticity	Source of stem cell
Totipotent	Each cell can develop into a new individual	Cells from early (1-3 days) embryos
Pluripotent	Cells can form any (over 200) cell types	Some cells of blastocyst (5-14 days)
Multipotent	Cells differentiated, but can form a number of other tissues	Fetal tissue, cord blood and post natal stem cells including dental pulp stem cells

V. PULP STEM CELLS

The dental pulp contains a population of stem cells, called pulp stem cells[11,12]or, in the case of immature teeth, stem cells from human exfoliated deciduous teeth (SHED).[13]. Sometimes pulp stem cells are called odontoblastoid cells, because these cells appear to synthesize and secrete dentin matrix like the odontoblast cells they replace[14].

Odontoblasts are postmitotic terminally differentiated cells, and cannot proliferate to replace subjacent irreversibly injured odontoblasts [15]. It was proposed that the cells within the subodontoblast cell–rich layer or zone of Hohl adjacent to the odontoblasts[16] differentiate into odontoblastoids.

One of the most significant obstacles to overcome in creating replacement pulp tissue for use in regenerative endodontics is to obtain progenitor pulp cells that will continually divide and produce cells or pulp tissues that can be implanted into root canal systems.

VI. GROWTH FACTORS

Growth factors are proteins that bind to receptors on the cell and induce cellular proliferation or differentiations. Many growth factors are quite versatile, stimulating cellular division in numerous cell types, while others are more cell specific. Bone morphogenic proteins (BMPs) are important growth factors required in tooth development and regeneration.

Recombinant BMP-2,-4,-7 induce formation of reparative dentin *in vivo* (Nakashima, 1994).

The application of recombinant human insulin like growth factor-1 together with collagen has been found to induce complete dentin bridging and tubular dentin formation (Lovschall et al, 2001). This indicates the potential of adding growth factors before pulp capping or incorporating them into restorative and endodontic materials to stimulate dentin and pulp regeneration. The therapeutic effect of calcium hydroxide may be because of its extraction of growth factors from dentin matrix (Smith et al, 1995). Once released, these growth factors may play key roles in signaling many of the events of tertiary dentinogenesis, a response of pulp dentin repair.

Data suggest that FGF2 plays a role not only as a differentiation inducing factor in the injury repair process of pulpal tissue but also as a positive regulator of chemokine expression, which may help in tissue engineering and pulp regeneration using Human DPSCs. However, the fate of odotoblastic or osteoblastic differentiation, effective local delivery for FGF2 interaction of chemotactic and odotogenic factor limitations need to be overcome (Kim et al, 2010).

Ability of MTA to induce useful cellular response to achieve suitable tissue wound healing is by promoting by adhesion, supporting cellular proliferation and by inducing migration of human mesenchymal stem cells. Mesenchymal stem cells are usually involved in tissue and bone remodeling, and local environment is thought to play an important role in the commitment and differentiation of mesenchymal derived stem cells (D'Anto et al, 2010).

VII. SCAFFOLD

The scaffold provides a physico-chemical and biological three dimensional micro environment for cell growth and differentiation, promoting cell adhesion and migration.

The scaffold serves as a carrier for morphogen in protein therapy and for cells in cell therapy.

Types of scaffolds:

(a) Biological or Natural eg. Collagen, Glycosaminoglycan
(b) Artificial or Synthetic eg. Poly lactic acid (PLA) Poly glycolic acid (PGA), Poly ethylene glycol

(PEG), Arginine, Hydroxyapatite, Tricalcium Phoshate.

VIII. POTENTIAL TECHNOLOGIES FOR REGENERATIVE ENDODONTICS

Several major areas of research that might have application in the development of regenerative endodontic techniques:

These techniques are the followings:

(a) root canal revascularization via blood clotting,
(b) postnatal stem cell therapy,
(c) pulp implantation,
(d) scaffold implantation,
(e) injectable scaffold delivery,
(f) three-dimensional cell printing, and
(g) gene delivery.

These regenerative endodontic techniques are based on the basic tissue engineering principles already described and include specific consideration of cells, growth factors, and scaffolds.

IX. ROOT CANAL REVASCULARIZATION VIA BLOOD CLOTTING

Several case reports have documented revascularization of necrotic root canal systems by disinfection followed by establishing bleeding into the canal system via over instrumentation [17-19].

An important aspect of these cases is the use of intracranial irrigants (NaOCl and chlorhexdine) with placement of antibiotics (e.g. a mixture of ciprofloxacin, metronidazole, and minocycline paste) for several weeks. This particular combination of antibiotics effectively disinfects root canal systems[20—22] and increases revascularization of avulsed and necrotic teeth[23,24] suggesting that this is a critical step in revascularization. The selection of various irrigants and medicaments is worthy of additional research, because these materials may confer several important effects for regeneration in addition to their antimicrobial properties. There are several advantages to a revascularization approach:

First, this approach is technically simple and can be completed using currently available instruments and medicaments without expensive biotechnology. Second, the regeneration of tissue in root canal systems by a patient's own blood cells avoids the possibility of immune rejection and pathogen transmission from replacing the pulp with a tissue engineered construct.

However, several concerns need to be addressed in prospective research. First, the case reports of a blood clot having the capacity to regenerate pulp tissue are exciting, but caution is required because the source of the regenerated tissue has not been identified. Generally, tissue engineering does not rely on blood clot formation, because the concentration and composition of cells trapped in the fibrin clot is unpredictable. This is a critical limitation to a blood clot revascularization approach, because tissue engineering is founded on the delivery of effective concentrations and compositions of cells to restore function.

On the other hand, some aspects of this approach may be useful; plasma-derived fibrin clots are being used for development as scaffolds in several studies[25].

Second, enlargement of the apical foramen is necessary to promote vascularizaton and to maintain initial cell viability via nutrient diffusion. Related to this point, cells must have an available supply of oxygen; therefore, it is likely that cells in the coronal portion of the root canal system either would not survive or would survive under hypoxic conditions before angiogenesis. Interestingly, endothelial cells release soluble factors under hypoxic conditions that promote cell survival and angiogenesis, whereas other cell types demonstrate similar responses to low oxygen availability[26-30].

X. POSTNATAL STEM CELL THERAPY

The simplest method to administer cells of appropriate regenerative potential is to inject postnatal stem cells into disinfected root canal systems after the apex is opened. Postnatal stem cells can be derived from multiple tissues, including skin, buccal mucosa, fat, and bone[31].

A major research obstacle is identification of a postnatal stem cell source capable of differentiating into the diverse cell population found in adult pulp (e.g., fibroblasts, endothelial cells, odontoblasts). Technical obstacles include the development of methods for harvesting and any necessary ex vivo methods required to purify and/or expand cell numbers sufficiently for regenerative endodontic applications. There are several advantages to an approachusing postnatal stem cells.

First, autogenous stem cells are relatively easy to harvest and to deliver by syringe, and the cells have the potential to induce new pulp regeneration. Second, this approach is already used in regenerative medical applications, including bone marrow replacement, and a recent review has described several potential endodontic applications[4].

However, there are several disadvantages to a delivery method of injecting cells.

First, the cells may have low survival rates.
Second, the cells might migrate to different locations within the body[32], possibly leading to aberrant patterns of mineralization.

A solution for this latter issue may be to apply the cells together with a fibrin clot or other scaffold material. This would help to position and maintain cell localization. In general, scaffolds, cells, and bioactive signaling molecules are needed to induce stem cell differentiation into a dental tissue type[33].

XI. PULP IMPLANTATION

The majority of invitro cell cultures grow as a single monolayer attached to the base of culture flasks. However, some stem cells do not survive unless they are grown on top of a layer of feeder cells[34].

In all of these cases, the stem cells are grown in two dimensions. In theory, to take two-dimensional cell cultures and make them three-dimensional, the pulp cells can be grown on biodegradable membrane filters. Many filters will be required to be rolled together to form a three dimensional pulp tissue, which can be implanted into disinfected root canal systems.

The advantages of this delivery system are that the cells are relatively easy to grow on filters in the laboratory. The growth of cells on filters has been accomplished for several decades, as this is how the cytotoxicity of many test materials is evaluated[35].Moreover, aggregated sheets of cells are more stable than dissociated cells administered by injection into empty root canal systems.

The potential problems associated with the implantation of sheets of cultured pulp tissue is that specialized procedures may be required to ensure that the cells properly adhere to root canal walls. Sheets of cells lack vascularity, so only the apical portion of the canal systems would receive these cellular constructs, with coronal canal systems filled with scaffolds capable of supporting cellular proliferation[36].

XII. SCAFFOLD IMPLANTATION

To create a more practical endodontic tissue engineering therapy, pulp stem cells must be organized into a three-dimensional structure that can support cell organization and vascularization. This can be accomplished using a porous polymer scaffold seeded with pulp stem cells. A scaffold should contain growth factors to aid stem cell proliferation and differentiation, leading to improved and faster tissue development[37]. The scaffold may also contain nutrients promoting cell survival and growth[38] and possibly antibiotics to prevent any bacterial in-growth in the canal systems. The engineering of nanoscaffolds may be useful in the delivery of pharmaceutical drugs to specific tissues[39]. The types of scaffold materials available are natural or synthetic, biodegradable or permanent.

The synthetic materials include polylactic acid (PLA), polyglycolic acid (PGA), and polycaprolactone (PCL), which are all common polyester materials that degrade within the human body[40]. These scaffolds have all been successfully used for tissue engineering applications because they are degradable fibrous structures with the capability to support the growth of various different stem cell types. The principal drawbacks are related to the difficulties of obtaining high porosity and regular pore size. This has led researchers to concentrate efforts to engineer scaffolds at the nanostructural level to modify cellular interactions with the scaffold[41].

XIII. INJECTABLE SCAFFOLD DELIVERY

Rigid tissue engineered scaffold structures provide excellent support for cells used in bone and other body areas where the engineered tissue is required to provide physical support.[42]. However, in root canal systems a tissue engineered pulp is not required to provide structural support of the tooth. This will allow tissue engineered pulp tissue to be administered in a soft three-dimensional scaffold matrix, such as a polymer hydrogel.

Hydrogels are injectable scaffolds that can be delivered by syringe. Hydrogels have the potential to be noninvasive and easy to deliver into root canal systems. In theory, the hydrogel may promote pulp regeneration by providing a substrate for cell proliferation and differentiation into an organized tissue structure.

Past problems with hydrogels included limited control over tissue formation and development, but advances in formulation have dramatically improved their ability to support cell survival. Despite these advances, hydrogels at are at an early stage of research, and this type of delivery system, although promising, has yet to be proven to be functional in vivo. To make hydrogels more practical, research is focusing on making them photopolymerizable to form rigid structures once they are implanted into the tissue site

XIV. THREE-DIMENSIONAL CELL PRINTING

The final approach for creating replacement pulp tissue may be to create it using a three-dimensional cell printing technique. In theory, an ink-jet-like device is used to dispense layers of cells suspended in a hydrogel to recreate the structure of the tooth pulp tissue. The three-dimensional cell printing technique can be used to precisely position cells, and this method has the potential to create tissue constructs that mimic the

natural tooth pulp tissue structure. The ideal positioning of cells in a tissue engineering construct would include placing odontoblastoid cells around the periphery to maintain and repair dentin, with fibroblasts in the pulp core supporting a network of vascular and nerve cells.

Theoretically, the disadvantage of using the three-dimensional cell printing technique is that careful orientation of the pulp tissue construct according to its apical and coronal asymmetry would be required during placement into cleaned and shaped root canal systems.

However, early research has yet to show that three-dimensional cell printing can create functional tissue in vivo.

XV. GENE THERAPY

A recent review has discussed the use of gene delivery in regenerative endodontics[4].

One use of gene delivery in endodontics would be to deliver mineralizing genes into pulp tissue to promote tissue mineralization.

However, a literature search indicates there has been little or no research in this field, except for the work of Rutherford. He transfected ferret pulps with cDNA-transfected mouse BMP-7 that failed to produce a reparative response, suggesting that further research is needed to optimize the potential of pulp gene therapy.

Moreover, potentially serious health hazards exist with the use of gene therapy; these arise from the use of the vector (gene transfer) system, rather than the genes expresse[43].

XVI. SUMMARY OF THE BARRIERS TO BE ADDRESSED TO PERMIT THE INTRODUCTION OF REGENERATIVE ENDODONTICS

1. Disinfection and shaping of root canals in a fashion to permit regenerative endodontics.
   Chemomechanical debridement — cleaning and shaping root canals
   Irrigants — 6% sodium hypochlorite and 2% chlorhexidine gluconate and alternatives
   Medicaments — Calcium hydroxide, triple antibiotics, MTAD, and alternatives.
2. Creation of replacement pulp-dentin tissues
   — Pulp revascularization by apex Instrumentation
   — Stem cells; allogenic, autologous;Xenogenic; umbilical cord sources
   — Growth factors; BMP-2, -4, -7; TGF-B1,-B2,-B3 among others
   — Gene therapy; identification of mineralizing genes
   — Tissue engineering; cell culture, scaffolds, hydrogels
3. Delivery of replacement pulp-dentin tissues
   Surgical implantation method Injection site
4. Dental restorative materials
   — Improve the quality of sealing between restorative materials and dentin.
   — Ensure long-term sealing to prevent recurrent pulpitis
5. Measuring appropriate clinical outcomes
   — Vascular blood flow
   — Mineralizing odontoblastoid cells
   — Intact afferent innervations
   — Lack of signs or symptoms

XVII. FUTURE DIRECTION

There is no unanimity of opinion concerning the usefulness of dental pulp capping. Although reparative dentin provides a physical barrier and protects the pulp, it has some limitations in the integrity.

When the pulp is exposed by caries, acute localized inflammation and liquefaction necrosis can be observed under the exposure site. It has been postulated that to preserve the remaining healthy pulp, this infected, necrotic, and disintegrated pulp tissue need to be removed. The complete restoration of the physiologic, structural, and mechanical integrity of the native dentin-pulp complex is the ultimate goal of endodontic treatment. Regeneration of pulp tissue in a necrotic infected tooth with apical periodontitis might be possible if the apex shows opening of more than 1.1 mm or apicoectomized, and if the tooth is replanted within 45 min., and if tooth is soaked in doxycycline or minocycline before replantation to be effectively disinfected.The blood clot created in the canal acts as a matrix for the growth of new tissue into the root canal space . An interesting question, the origin of the new pulp tissue still remains to be answered.

Further systematic studies with stem/progenitor cells, morphogens, novel scaf- folds, and effective disinfecting are required for regenerative therapy in apical periodontitis. The regenerative therapy will revolutionize the future endodontics with the synergistic confluence of advances in signaling pathways underlying morphogenesis and lineage of stem/progenitor cells by morphogens such as BMPs and synthetic scaffolds.

XVIII. CONCLUSION

Stem cells derived from all sources hold immense medical promises. Stem cell therapies have virtually unlimited medical and dental applications.Tissue engineering using the triad of dental pulp stem cells, morphogens and scaffolds may provide an innovative and biologically based approach for generation of clinical materials and treatment of dental diseases. The challenges of introducing endodontic tissue engineered therapies are substantial; the potential benefits to patients and the profession are ground breaking. One of the most challenging aspects of developing a regenerative endodontic therapy is to understand how the various component procedures can be optimized and integrated to produce the outcome of a regenerated pulp-dentin complex. The future development of regenerative endodontic procedures will require a comprehensive research program directed at each of these components and their application to our patients. The authors believe that regenerative endodontics is an inevitable therapy, and they call for action from scientists, funding agencies, and the endodontic profession to pool resources to hasten its development. The unleashed potential of regenerative endodontics may benefit millions of patients each year.

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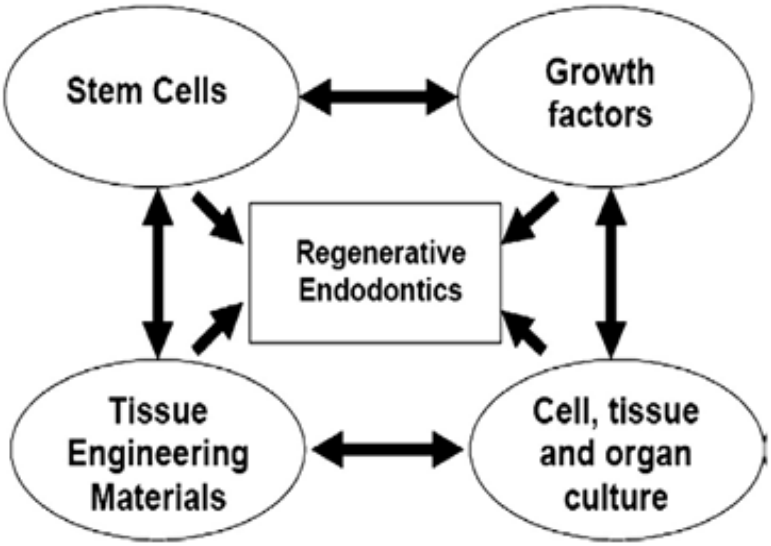

Fig 1. The major domains of research required to develop Regenerative endodontic procedures

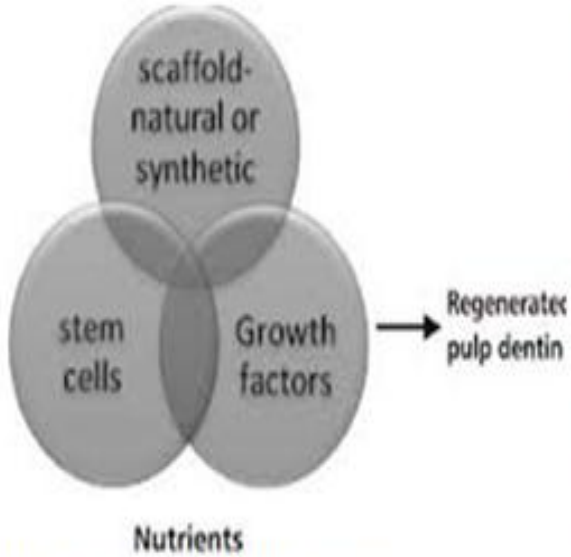

Fig .2: Tissue Engineering Triad

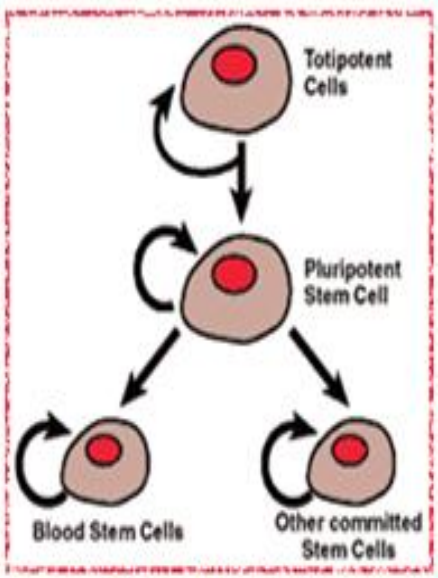

Fig. 3: Stem cells

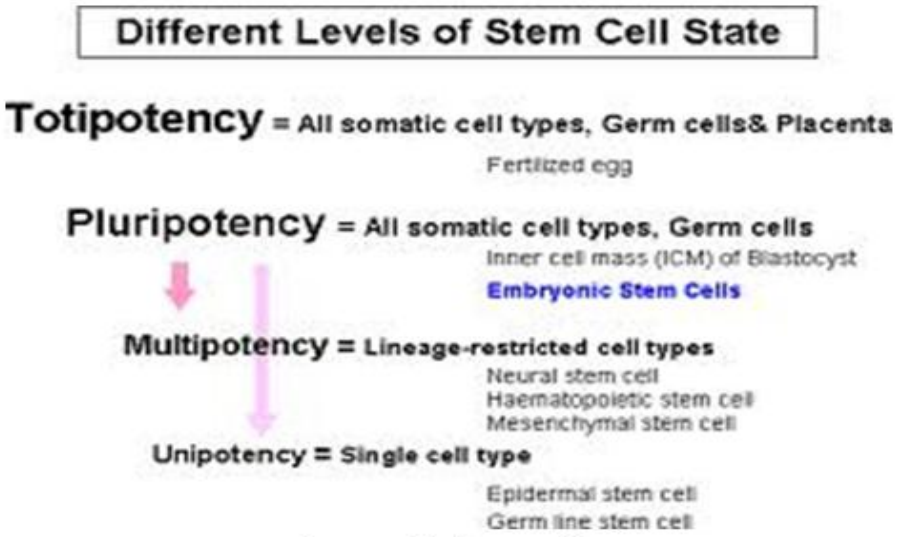

Fig.4: Levels of Stem cells

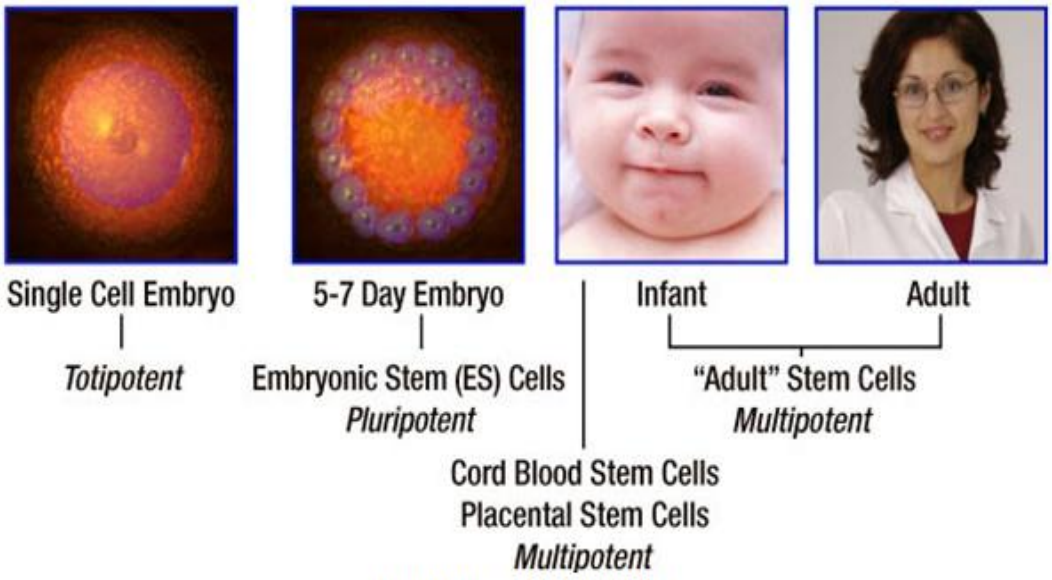

Fig.5: Phases of stem cells