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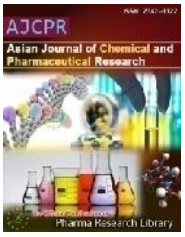

Review Article Open Access

Review on Stem Cell Therapy

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A B S T R A C T

Stem cells have the ability to differentiate into specific cell types. The two defining characteristics of a stem cell are perpetual self-renewal and the ability to differentiate into a specialized adult cell type. There are two major classes of stem cells: pluripotent that can become any cell in the adult body and multipotent those are restricted to becoming a more limited population of cells. Cell sources, characteristics, differentiation and therapeutic applications are discussed. Stem cells have great potential in tissue regeneration and repair but much still needs to be learned about their biology, manipulation and safety before their full therapeutic potential can be achieved. While the regeneration of a lost tissue is known to mankind for several years, 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. Initial evidence from pioneering studies has documented the likely breakthrough that stem cells offer for various life-threatening diseases that have so far defeated modern medical care. The evidence gathered so far has propelled many elegant studies exploring the role of stem cells and their manifold dental applications. This review takes you on a sojourn 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: Stem cell, human body, Cell sources

A R T I C L E I N F O

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1. Introduction

Stem cells have the ability to build every tissue in the human body, hence have great potential for future therapeutic uses in tissue regeneration and repair. In order for cells to fall under the definition of "stem cells," they must display two essential characteristics. First, stem cells must have the ability of unlimited self-renewal to produce progeny exactly the same as the originating cell. This trait is also true of cancer cells that divide in an uncontrolled manner whereas stem cell division is highly regulated. Therefore, it is important to note the additional requirement for stem cells; they must be able to give rise to a specialized cell type that becomes part of the healthy animal.

The general designation, "stem cell" encompasses many distinct cell types. Commonly, the modifiers, "embryonic," and "adult" are used to distinguish stem cells by the developmental stage of the animal from which they come, but these terms are becoming insufficient as new research has discovered how to turn fully differentiated adult cells back into embryonic stem cells and, conversely, adult stem cells, more correctly termed "somatic" stem cells meaning "from the body", are found in the fetus, placenta, umbilical cord blood and infants. Therefore, this review will sort stem cells into two categories based on their biologic properties - pluripotent stem cells and multipotent stem cells. Their sources, characteristics, differentiation and therapeutic applications are discussed.

Pluripotent stem cells are so named because they have the ability to differentiate into all cell types in the body. In natural development, pluripotent stem cells are only present for a very short period of time in the embryo before differentiating into the more specialized multipotent stem cells that eventually give rise to the specialized tissues of the body. These more limited multipotent stem cells come in several subtypes: some can become only cells of a particular germ line (endoderm, mesoderm, ectoderm) and others, only cells of a particular tissue. In other words, pluripotent cells can eventually become any cell of the body by differentiating into multipotent stem cells that themselves go through a series of divisions into even more restricted specialized cells. The sojourn of science has unraveled and understood that the secret of life lies in the "DNA," thanks to Sir James Watson and Crick for their epoch making a historic discovery. In our endeavor to demystify the DNA, we have realized that scientific discoveries in and molecular biology have truly revolutionized our collective understanding of the biological processes that could greatly impact and dramatically change our lives in the future.

In the new millennium, where biology and biotechnology have replaced chemistry, we are exploring "biological solutions to biological problems." Owing to the extraordinary advances taking place in the field of cellular and molecular biology, we are on the verge of a paradigm shift, evolving from offering simple mechanical care to consider biological solutions to health promotion, risk assessment, diagnosis, treatment, and even prognosis.

Although stem cell technology is just emerging, the regeneration of body parts is hardly a new concept. The regenerative capability of a living creature was recorded as early as 330 BC, when Aristotle observed that a lizard could grow back the lost tip of its tail. Since then, there have been slow but steady attempts at understanding the regenerative capabilities of human being and it is only in the last decade that we have seen an information explosion in the area of stem cell research. Stem cells are likely to revolutionize the entire health care delivery. The time is certainly ripe for all of us to familiarize ourselves with the following: what are stem cells, their characteristics, their potential applications, current research translating to therapy, and possible barriers of its application from the bench to the bedside/chair.

This primer on stem cells is intended for anyone who wishes to learn more about the biological properties of stem cells, the important questions about stem cells that are the focus of scientific research, and the potential use of stem cells in research and in treating disease. The primer includes information about stem cells derived from embryonic and non-embryonic tissues. Much of the information included here is about stem cells derived from human tissues, but some studies of animal-derived stem cells are also described

2. Sources of Stem cells

Pluripotent: Pluripotent stem cells being used in research today mainly come from embryos, hence the name, "embryonic stem cells". Pre-implantation embryos a few days old contain only 10-15% pluripotent cells in the "inner cell mass". Those pluripotent cells can be isolated, then cultured on a layer of "feeder" cells which provide unknown cues for many rounds of proliferation while sustaining their pluripotency.

Recently, two different groups of scientists induced adult cells back into the pluripotent state by molecular manipulation to yield "induced pluripotent stem cells" (iPS) that share some of the same characteristics as embryonic stem cells such as proliferation, morphology and gene expression (in the form of distinct surface markers and proteins being expressed).[4-8] Both groups used retroviruses to carry genes for transcription factors into the adult cells. These genes are transcribed and translated into proteins that regulate the expression of other genes designed to reprogram the adult nucleus back into its embryonic state. Both introduced the embryonic transcription factors known as Sox2 and Oct4. One group also added Klf4 and c-Myc[4], and the other group added Lin28 and Nanog.[6] Other combinations of factors would probably also work, but,

unfortunately, neither the retroviral carrier method nor the use of the oncogenic transcription factor c-Myc are likely to be approved for human therapy. Consequently, a purely chemical approach to deliver genes into the cells, and safer transcription factors are being tried. Results of these experiments look promising.[9]

3. Multipotent

Multipotent stem cells may be a viable option for clinical use. These cells have the plasticity to become all the progenitor cells for a particular germ layer or can be restricted to become only one or two specialized cell types of a particular tissue. The multipotent stem cells with the highest differentiating potential are found in the developing embryo during gastrulation (day 14-15 in humans, day 6.5-7 in mice). These cells give rise to all cells of their particular germ layer, thus, they still have flexibility in their differentiation capacity. They are not pluripotent stem cells because they have lost the ability to become cells of all three germ layers. On the low end of the plasticity spectrum are the unipotent cells that can become only one specialized cell type such as skin stem cells or muscle stem cells. These stem cells are typically found within their organ and although their differentiation capacity is restricted, these limited progenitor cells play a vital role in maintaining tissue integrity by replenishing aging or injured cells. There are many other sub-types of multipotent stem cells occupying a range of differentiation capacities. For example, multipotent cells derived from the mesoderm of the gastrula undergo a differentiation step limiting them to muscle and connective tissue; however, further differentiation results in increased specialization towards only connective tissue and so on until the cells can give rise to only cartilage or only bone.

Multipotent stem cells found in bone marrow are best known, because these have been used therapeutically since the 1960's (their potential will be discussed in greater detail in a later section). Recent research has found new sources for multipotent stem cells of greater plasticity such as the placenta and umbilical cord blood. Further, the heart, until recently considered void of stem cells, is now known to contain stem cells with the potential to become cardiac myocytes. Similarly, neuro-progenitor cells have been found within the brain. The cardiac stem cells are present in such small numbers, that they are difficult to study and their function has not been fully determined. The second review in this series will discuss their potential in greater detail.

Unique Properties of stem cells:

Stem cells differ from other kinds of cells in the body. All stem cells—regardless of their source—have three general properties: they are capable of dividing and renewing themselves for long periods; they are unspecialized; and they can give rise to specialized cell types. *Stem cells are capable of dividing and renewing themselves for long periods*. Unlike muscle cells, blood cells, or nerve cells—which do not normally replicate themselves—stem cells may replicate many times, or proliferate. A starting population of stem cells that proliferates for many months in the laboratory can yield millions of cells. If the resulting cells continue to be unspecialized, like the parent stem cells, the cells are said to be capable of long-term self-renewal.

- Scientists are trying to understand two fundamental properties of stem cells that relate to their long-term self-renewal
- Why can embryonic stem cells proliferate for a year or more in the laboratory without differentiating, but most adult stem cells cannot;and What are the factors in living organisms that normally regulate stem cell proliferation and self-renewal

Discovering the answers to these questions may make it possible to understand how cell proliferation is regulated during normal embryonic development or during the abnormal cell division that leads to cancer. Such information would also enable scientists to grow embryonic and non-embryonic stem cells more efficiently in the laboratory.

The specific factors and conditions that allow stem cells to remain unspecialized are of great interest to scientists. It has taken scientists many years of trial and error to learn to derive and maintain stem cells in the laboratory without them spontaneously differentiating into specific cell types. For example, it took two decades to learn how to grow human embryonic stem cells in the laboratory following the development of conditions for growing mouse stem cells. Likewise, scientists must first understand the signals that enable a non-embryonic (adult) stem cell population to proliferate and remain unspecialized before they will be able to grow large numbers of unspecialized adult stem cells in the laboratory.

Stem cells are unspecialized. One of the fundamental properties of a stem cell is that it does not have any tissue-specific structures that allow it to perform specialized functions. For example, a stem cell cannot work with its neighbors to pump blood through the body (like a heart muscle cell), and it cannot carry oxygen molecules through the bloodstream (like a red blood cell). However, unspecialized stem cells can give rise to specialized cells, including heart muscle cells, blood cells, or nerve cells.

Stem cells can give rise to specialized cells. When unspecialized stem cells give rise to specialized cells, the process is called differentiation. While differentiating, the cell usually goes through several stages, becoming more specialized at each step. Scientists are just beginning to understand the signals inside and outside cells that trigger each step of the differentiation process. The internal signals are controlled by a cell's genes, which are interspersed across long strands of DNA and carry coded instructions for all cellular structures and functions. The external signals for cell differentiation include chemicals secreted by other cells, physical contact with neighboring cells, and certain molecules in the microenvironment. The interaction of signals during differentiation causes the cell's DNA to

acquire epigenetic marks that restrict DNA expression in the cell and can be passed on through cell division.

Many questions about stem cell differentiation remain. For example, are the internal and external signals for cell differentiation similar for all kinds of stem cells? Can specific sets of signals be identified that promote differentiation into specific cell types? Addressing these questions may lead scientists to find new ways to control stem cell differentiation in the laboratory, thereby growing cells or tissues that can be used for specific purposes such as cell-based therapies or drug screening.

Adult stem cells typically generate the cell types of the tissue in which they reside. For example, a blood-forming adult stem cell in the bone marrow normally gives rise to the many types of blood cells. It is generally accepted that a blood-forming cell in the bone marrow—which is called a hematopoietic stem cell—cannot give rise to the cells of a very different tissue, such as nerve cells in the brain. Experiments over the last several years have purported to show that stem cells from one tissue may give rise to cell types of a completely different tissue. This remains an area of great debate within the research community. This controversy demonstrates the challenges of studying adult stem cells and suggests that additional research using adult stem cells is necessary to understand their full potential as future therapies.

Stem cells and their Characteristics

They are unspecialized cells with an extraordinary ability to self-renew, capable of differentiating into one or more specialized cell types playing a crucial role in homeostasis and tissue repair. When called into action following an injury, a stem cell self-renews – undergoes cell division and gives rise to one daughter stem cell and one progenitor cell. A progenitor cell is an intermediate cell type formed before it achieves a fully differentiated state. It is regarded as committed to differentiating along a particular cellular developmental pathway of stem cells:

Stem cell → Stem cell + Progenitor cell → Differentiated cell

Based on their origin, stem cells are categorized either as embryonic stem cells (ESCs) or as postnatal stem cells/somatic stem cells/adult stem cells (ASCs).

Characteristics:

1. Totipotency: generate all types of cells including germ cells (ESCs).
2. Pluripotency: generate all types of cells except cells of the embryonic membrane.
3. Multipotency: differentiate into more than one mature cell (MSC).
4. Self-renewal: divide without differentiation and create everlasting supply.
5. Plasticity: MSCs have plasticity and can undergo differentiation. The trigger for plasticity is stress or tissue injury which upregulates the stem cells and releases chemoattractants and growth factors.

Among the types of differentiation are:

1. Direct differentiation: a specific type of cell in a special niche developed in a multistep unidirectional pathway (e.g., MSCs differentiating into osteoblasts/fibroblasts).
2. Transdifferentiation: direct conversion of one cell type to another different cell type (e.g., blood cells into brain cells and vice versa).
3. Dedifferentiation: a unipotent stem cell becoming a multipotent one.
4. Cell fusion: a stem cell fusing with a somatic cell resulting in another lineage (e.g., ESCs fuse in vitrowith HSCs and neuronal cells).

Embryonic stem cells

Embryonic stem cells (ESCs) are derived from embryos that are 2–11 days old called blastocysts. They are best grown from supernumerary embryos obtained from in vitro fertilization centers. They are totipotent – cells virtually capable of differentiating into any type of cell including the germ cell. ESCs are considered immortal as they can be propagated and maintained in an undifferentiated state indefinitely. These stem cells have the highest potential to regenerate and repair diseased tissue and organs in the body.[1,2] However, the therapeutic benefit of ESCs is bogged by a controversy owing to the belief that the process of extraction of stem cells from an embryo destroys the embryo itself and some view this as taking life, thereby, raising moral and ethical concerns. Further, it is difficult to control the growth and differentiation of the embryonic stem cell posing risk of tumorogenicity and teratoma formation. While research is on to overcome some of these shortfalls as of now, ESCs are not so far used therapeutically and have only remained an excellent platform for research.

Adult stem cells

Adult stem cells are found in most adult tissues. They are multipotent – capable of differentiating into more than one cell type but not all cell types. The plasticity of an adult stem cell is described as its ability to expand beyond its potential irrespective of the parent cell from which it is derived. For example, dental pulp stem cells not only develop into tooth tissue but also have the ability to differentiate into neuronal tissue. Depending on their origin, adult stem cells can be further classified as hemopoetic stem cells (HSCs) and mesenchymal stem cells (MSCs). HSCs are obtained either from cord blood or peripheral blood. MSCs are those that originate from the mesoderm layer of the fetus and in the adult reside in a variety of tissues such as the bone marrow stem cells (BMSCc), limbal stem cells, hepatic stem cells, dermal stem cells, etc.

Stem cells have also been isolated from orofacial tissues which include adult tooth pulp tissue, pulp tissue of deciduous teeth, periodontal ligament, apical papilla, and buccal mucosa. Gronthos et al. have isolated stem cells from adult human dental pulp (DPSCs) that exhibit a similar immunophenotype to bone marrow stem cells. Stem cells from human exfoliated deciduous teeth (SHED) represent a unique population of multipotent stem cells that are easily accessible and are more immature in the cell hierarchy than the adult pulp stem cells. Using a similar methodology, multipotent stem cells from the human periodontal ligament (PDLCs) have also been described.

Recently, a new population of mesenchymal stem cells (MSCs) residing in the apical papilla of incompletely developed teeth (SCAP) have been isolated and demonstrated in elegant studies.

4. Stem Cell therapy

Pluripotent stem cells

Pluripotent stem cells have not yet been used therapeutically in humans because many of the early animal studies resulted in the undesirable formation of unusual solid tumors, called teratomas. Teratomas are made of a mix of cell types from all the early germ layers. Later successful animal studies used pluripotent cells modified to a more mature phenotype which limits this proliferative capacity. Cells derived from pluripotent cells have been used to successfully treat animals. For example, animals with diabetes have been treated by the creation of insulin-producing cells responsive to glucose levels. Also, animals with acute spinal cord injury or visual impairment have been treated by creation of new myelinated neurons or retinal epithelial cells, respectively. Commercial companies are currently in negotiations with the FDA regarding the possibility of advancing to human trials. Other animal studies have been conducted to treat several maladies such as Parkinson's disease, muscular dystrophy and heart failure. Scientists hope that stem cell therapy can improve cardiac function by integration of newly formed beating cardiac myocytes into the myocardium to produce greater force. Patches of cardiac myocytes derived from human embryonic stem cells can form viable human myocardium after transplantation into animals, with some showing evidence of electrical integration. Damaged rodent hearts showed slightly improved cardiac function after injection of cardiac myocytes derived from human embryonic stem cells. The mechanisms for the gain in function are not fully understood but it may be only partially due to direct integration of new beating heart cells. It is more likely due to paracrine effects that benefit other existing heart cells.

Multipotent stem cells

Multipotent stem cells harvested from bone marrow have been used since the 1960's to treat leukemia, myeloma and lymphoma. Since cells there give rise to lymphocytes, megakaryocytes and erythrocytes, the value of these cells is easily understood in treating blood cancers. Recently, some progress has been reported in the use of cells derived from bone marrow to treat other diseases. For example, the ability to form whole joints in mouse models[27] has been achieved starting with mesenchymal stem cells that give rise to bone and cartilage. In the near future multipotent stem cells are likely to benefit many other diseases and clinical conditions. Bone marrow-derived stem cells are in clinical trials to remedy heart ailments. This is discussed in detail in the next review of this series.

Pluripotent vs. Multipotent

Pluripotent and multipotent stem cells have their respective advantages and disadvantages. The capacity of pluripotent cells to become any cell type is an obvious therapeutic advantage over their multipotent kin. Theoretically, they could be used to treat diseased or aging tissues in which multipotent stem cells are insufficient. Also, pluripotent stem cells proliferate more rapidly so can yield higher numbers of useful cells. However, use of donor pluripotent stem cells would require immune suppressive drugs for the duration of the graft while use of autologous multipotent stem cells (stem cells from ones' self) would not. This ability to use one's own cells is a great advantage of multipotent stem cells. The immune system recognizes specific surface proteins on cells/objects that tell them whether the cell is from the host and is healthy. Autologous, multipotent stem cells have the patient's specific surface proteins that allow it to be accepted by the host's immune system and avoid an immunological reaction. Pluripotent stem cells, on the other hand, are not from the host and therefore, lack the proper signals required to stave off rejection from the immune system. Research is ongoing trying to limit the immune response caused by pluripotent cells and is one possible advantage that iPS cells may have.

Potential Applications in Medicine:

Stem cells are being explored for a variety of chronic debilitating diseases that have so far escaped remedial measures from traditional allopathic approaches with a hope that cell therapy would repair, repopulate, replace, and rewire tissues and organs regenerating hope and kindling confidence in such therapies.

Because of the overwhelming success of animal studies, numerous clinical trials are now going on world over. Various therapeutic programs in either pilot or proof of concept studies are exploring the role of cell replacement therapy under conditions like Parkinson's disease, spinal cord injury, heart failure, hematological disease, cancer, arthritis, diabetes, and peripheral vascular disease. Interim results from the pilot studies are encouraging and have led the US FDA to permit Phase III clinical trials for acute and steroid refractory graft versus host disease and also Crohn's disease, Phase II clinical trials for the repair of heart tissue following a heart attack, the protection of pancreatic islet cells in patients with type 1 diabetes, and the repair of lung tissue in patients with chronic obstructive pulmonary disease. An injectable formulation of mesenchymal stem cells, for arthritis in the knee, is also being evaluated. Even in India, we are not far behind since Phase II proof of concept studies has been approved and is underway.

Stem-cell therapy for cardiac disease:

Heart failure is the leading cause of death worldwide, and current therapies only delay progression of the disease. Laboratory experiments and recent clinical trials suggest that cell-based therapies can improve cardiac function, and the implications of this for cardiac regeneration are causing great excitement. Bone-marrow-derived progenitor cells and other progenitor cells can differentiate into vascular cell types, restoring blood flow. More recently, resident cardiac stem cells have been shown to differentiate into multiple cell types present in the heart, including cardiac muscle cells, indicating that the heart is not terminally differentiated. These new findings have stimulated optimism that the progression of heart failure can be prevented or even reversed with cell-based therapy.

Stem cell therapy for human neurodegenerative disorders: Recent progress shows that neurons suitable for transplantation can be generated from stem cells in culture,

and that the adult brain produces new neurons from its own stem cells in response to injury. These findings raise hope for the development of stem cell therapies in human neurodegenerative disorders. Before clinical trials are initiated, we need to know much more about how to control stem cell proliferation and differentiation into specific phenotypes, induce their integration into existing neural and synaptic circuits, and optimize functional recovery in animal models closely resembling the human disease.

Stem cells for the treatment of neurological disorders:

Many common neurological disorders, such as Parkinson's disease, stroke and multiple sclerosis, are caused by a loss of neurons and glial cells. In recent years, neurons and glia have been generated successfully from stem cells in culture, fuelling efforts to develop stem-cell-based transplantation therapies for human patients. More recently, efforts have been extended to stimulating the formation and preventing the death of neurons and glial cells produced by endogenous stem cells within the adult central nervous system. The next step is to translate these exciting advances from the laboratory into clinically useful therapies

Potential applications in Dentistry:

The regenerative potential of adult stem cells obtained from various sources including dental tissues has been of interest for clinicians over the past years and most research is directed toward achieving the following:

- Regeneration of damaged coronal dentin and pulp
- Regeneration of resorbed root, cervical or apical dentin, and repair perforations
- Periodontal regeneration
- Repair and replacement of bone in craniofacial defects
- Whole tooth regeneration.

Regeneration of damaged coronal dentin and pulp

To this date, no restorative material has been able to mimic all physical and mechanical properties of tooth tissue. Furthermore, we have not been successful in providing an ideal solution to certain situations, such as an immature tooth with extensive coronal destruction and reversible pulpitis. If the regeneration of tooth tissue is possible in these situations, it facilitates physiologic dentin deposition that forms an integral part of the tooth thereby restoring structural integrity, minimizing interfacial failure, microleakage, and other consequent complications. Similarly, young permanent teeth that require apexogenesis or apexification are the perfect candidates for the regeneration of pulp as they allow completion of both vertical and lateral root development, improving the long-term prognosis. However, pulp regeneration in fully formed teeth may not be of great benefit, although there is sufficient evidence to say that a restored vital tooth serves longer than a root-canal-treated one. Pulp tissue regeneration involves either delivery of autologous /allogenic stem cells into the root canals or implantation of the pulp that is grown in the laboratory using stem cells. Both these techniques will have certain advantages and limitations that need further research.

A landmark study conducted by Gronthos *et al*. demonstrated both *in vitro* and *in vivo* in animals that dental pulp stem cells (DPSCs) were capable of forming ectopic dentin and associated pulp tissue. Batouli *et al*. used an *in vivo* stem cell transplantation system to investigate differential regulation mechanisms of bone marrow stromal stem cells (BMSCs) and DPSCs. DPSCs were found to be able to generate a reparative dentin-like tissue on the surface of human dentin *in vivo*. This study provided direct evidence to suggest that osteogenesis and dentinogenesis mediated by BMSCs and DPSCs, respectively, may be regulated by distinct mechanisms, leading to the different organization of the mineralized and nonmineralized tissues.

Periodontal regeneration:

Regenerating the periodontium has always been a high priority in craniofacial regenerative biology. Due to the complex structure of the periodontium (consisting of hard and soft tissues), its complete regeneration has always remained a challenge. All the current regenerative techniques such as autologous bone grafts, allografts, or alloplastic materials have limitations and cannot be used in all clinical situations. Therefore, a cell-mediated bone regeneration technique will be a viable therapeutic alternative. Kawaguchi *et al*. demonstrated that the transplantation of *ex vivo* expanded autologous MSCs can regenerate new cementum, alveolar bone, and periodontal ligament in class III periodontal defects in dogs. Going a step further, periodontal ligament cells cultured *in vitro* were successfully reimplanted into periodontal defects in order to promote periodontal regeneration by Hasegawa *et al*. A subsequent study by the same group reported a similar approach in humans. This study reported firm evidence that stem cells can be used to regenerate a tissue as complex as the periodontium.

Repair and regeneration of bone in craniofacial defects:

Craniofacial bone grafting procedures rely on autologous bone grafting, devitalized allogenic bone grafting (using bone from bone bank), and natural/synthetic osteoconductive biomaterials. Autologous bone grafting is limited by donor site morbidity and allogenic bone is often destroyed soon. A long-term outcome using biomaterials relies on their ability to encourage local cells to completely regenerate a defect and results are often not encouraging. If stem cells can be harvested in a scaffold and transplanted into a defect to regenerate the lost tissue, it can alleviate a lot of complications associated with the traditional techniques. Abukawa *et al*. used a novel scaffold design with a new fabrication protocol to generate an autologous tissue engineered construct which was used to repair a segmental mandibular defect. The technique promoted osteogenesis and enhanced penetration of bone with blood vessels thereby accelerating tissue regeneration. In a dog model, Yamada *et al*. showed that a mixture of MSCs and platelet-rich plasma improved bone implant contact and bone density in a mandibular defect. The development of new scaffold fabrication technologies has facilitated a successful repair of three dimensionally complex cranial defects. To further enhance the regenerative potential of MSCs, genetic engineering technologies have been utilized to extend the life of stem cells and to enhance osteogenesis. In summary, cell-derived therapy for the repair of osseous defects has been relatively successful and numerous clinical trials in human craniofacial defects are underway.

5. Whole tooth regeneration

A therapeutic option that was unthinkable a few years ago seems an achievable goal today. Even to this day, the replacement of missing teeth has limitations. Although, implants are a significant improvement over dentures and bridges, their fundamental limitation is the lack of natural structural relationship with the alveolar bone (absence of periodontal ligament). They rely on direct integration of bone on tooth surface which is indeed an unnatural relationship as compared with the natural tooth. Further, they are also associated with a lot of esthetic, functional, and surgical limitations that affect their prognosis. Ohazama *et al*. reported the reconstruction of murine teeth using cultured stem cells which when transferred into renal capsules resulted in the development of tooth structures and associated bone. Nakao *et al*. recently engineered teeth ectopically and transplanted them into an anthrotopic site in a mouse jaw. Sonayama *et al*. used SCAP and PDLSCs and formed a bioroot in mini pigs. SCAP and PDLSCs were seeded in a scaffold and implanted into the sockets of the lower jaw. Postchannels were precreated to leave space for postinsertion and 3 months later the bioroot was exposed and a porcelain crown was inserted. The bioroot developed, and had a natural relationship with the surrounding bone.

6. Conclusion

The promises of cures for human ailments by stem cells have been much touted but many obstacles must still be overcome. First, more human pluripotent and multipotent cell research is needed since stem cell biology differs in mice and men. Second, the common feature of unlimited cell division shared by cancer cells and pluripotent stem cells must be better understood in order to avoid cancer formation. Third, the ability to acquire large numbers of the right cells at the right stage of differentiation must be mastered. Fourth, specific protocols must be developed to enhance production, survival and integration of transplanted cells. Finally, clinical trials must be completed to assure safety and efficacy of the stem cell therapy. When it comes to stem cells, knowing they exist is a long way from using them therapeutically.

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