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Stem cells: The future of periodontal regeneration

Arif Siddique[1*], Surbhi Priyadarshi[2]
[1] Postgraduate Student, Department of Periodontics and Implantology, Teerthanker Mahaveer Dental College and Research Centre, Moradabad, Uttar Pradesh, India
[2] Postgraduate Student, Department of Public Health Dentistry, Teerthanker Mahaveer Dental College and Research Centre, Moradabad, Uttar Pradesh, India

Abstract
Nowadays, dentistry is taking great steps in the field of research and is bringing research into clinical application. The research on stem cells is providing a big hope for the possibility of periodontal regeneration. The stem cells have the property to grow at very fast rate. The stem cells can form specialized dentin, neuronal cells and bone. This property will definitely help in providing better and newer treatment options to the patient. They can help in treating destructed teeth, neuronal injury and can induce new bone formation. In this aspect, the role of stem cells becomes important for periodontal regeneration. The literature was searched using electronic data base from 1978 to 2019. The search was restricted to English language articles, and various *in vitro* and *in vivo* studies were included. Google search was also done.

Keywords: periodontal regeneration, periodontitis, stem cells

Introduction
Inflammatory disease of the periodontium is known as periodontitis. According to National survey 15% of population is suffering from generalized severe periodontistis [1].
For periodontal regeneration several methods have been applied previously like root conditioning, guided tissue regeneration, periodontal regeneration, bone graft, and the application of growth factor. However, these regenerative procedures have some limitations mainly in areas of periodontal defects at advance stages [2, 3]. This discussion reviews regenerative therapy in cases of periodontitis and current knowledge of stem cells.

Sources of the stem cells
The main sources of stem cells are-
1. Embryonic stem cells (ES) which are formed from the inner mass of the blastocyst in a 4 to 5 days embryo.
2. Embryonic germ cells (EG) which can be collected from the foetal tissue in the later stage of the development.
3. Adult stem cells can be derived from the mature tissues in adults [4].

Pluripotent stem cells are a very old concept. Embryonic cells of mouse were discovered in 1981 [5]. This provided a paradigm shift in the development of embryonic stem cells in human. Embryonic stem cells of humans may not provide all but most of the cell types of human body and this may provide a big help in field of cell therapy with a resource full of unlimited cells but this requires a deep understanding of properties of embryonic stem cells in human, only then they can be used for the purpose of clinical applications. Levenberg S *et al* told about direct derivation of embryonic germ cells from the mouse's primordial germ cell [5]. The development capacity of these germ cells was very similar to the embryonic stem cells with some differences in the gene expression. The ES cells of mouse provided a definition of generic requirements of these embryonic stem cells. It can be derived from pluripotent stem cell population and is stably diploid. It's karyotyping is normal in the invitro study and we can propagate it indefinitely in early embryonic state. It can be differentiated into multiple cells representating all the three germ layers, in both the *in vitro* and from teratomas after the grafting. It can form any cell type of the body when colonize in the host blastocyst like the germ cells.

Mesenchymal stem cells sources
Many studies have identified mesenchymal clonogenic stem cells from spleen, thymus and bone marrow [6]. Many studies have found mesenchymal clonogenic stem cells like cells from skin, placenta [7], Vasculature of the umbilical cord, adipose tissues, blood [8], tendons, blood vessels, skeletal muscle, synovium, synovial fluid, trabecular bone, articular cartilage and the periosteum. It can also be derived from dental tissues such as periodontal ligament, dental follicles, exfoliated human deciduous teeth, dental pulp [9] and apical papilla [10]. The relationship between different types of clonogenic mesenchymal stem cells is still not known. Mesenchymal precursors can be derived from differentiated embryonic stem cells [11] and pluripotent stem cells of human [12].

Generic criteria for pluripotent embryonic stem or embryonic germ cells
It is originated from pluripotent stem cell population and it maintains normalcy of the karyotype. It can be propagated in embryonic state indefinitely and they are immortal. The Clones culture derived from it has the capability of differentiation into extra embryonic tissues and the somatic cells with all the three germ layers in the invitro or in teratoma.

However these criteria till now are only met by embryonic stem cells and embryonic germ cells of the mouse only. Thus far, only mouse EG or ES cells meet these generic criteria. Embryonic stem cells of the mouse only meet some of these criterias, but not all.

Characteristics of mesenchymal stem cells

Mesenchymal stem cells are multipotent in nature as they have the ability, even in cases of clones of isolated cells, to differentiate into a different cells and tissue lineages. They differentiate along the specific mesenchymal lineages and remain in a silent undifferentiated state till the time the signals are provided to divide asymmetrically. They finally undergoes many replicative cycles more than the normal and fully differentiated cells [13].

Properties of the stem cells

All the pluripotent stem cells of the primate grows in rounded clumps and they have indistinct cell borders. They express activity of enzyme alkaline phosphatise. 4 types of isozymes of the enzyme alkaline phosphatise has been identified in humans. The non specific form of the tissues is expressed by the embryonic germ cells. Embryonic germ cells also express a enzyme which can be detected with the help of antibodies which react with germ cell or with the placental form. Feeder cell layer of the embryonic fibroblast of mouse is required by the pluripotent stem cells. This need can be substituted by LIF or related members of cytokine family. The pluripotent embryonic germ cells of humans, embryonic stem cells of humans and embryonic stem cells of rhesus monkey will not show any respond to the LIF in such fashion.

Stem cells in dental and periodontal tissues

Recently regenerative medicine has been greatly advanced due to the manipulation and identification of the stem cells and this has contributed for the development of the tissue engineering based clinical therapies. The delivery of the ex-vivo progenitor expanded population mobilization of the progenitor endogenous cells which are capable to differentiate and proliferate into required tissues is the critical need of the tissue engineering. Periodontal ligament is having one of the highest turnover rates in our body and it is highly fibrous as well as vascular tissue. It consists of following cells like cementoblasts, fibroblast, osteoblasts, epithelial and endothelial cells. It also has a population of "progenitor stem cells" that has some of the characteristics of mesenchymal stem cells. The dental stem cells in humans which were isolated from third molar's dental pulp have characteristics similar to mesenchymal stem cells of the bone marrow. Stem cells of periodontal ligament can also give rise to clonogenic clusters which resembles fibroblasts and they can develop into osteoblast or adipocytes like cells and also in cementoblasts like cells *in vitro*. They can also develop into periodontal ligament and cementum like tissues *in vivo*. They have similarity with dental pulp stem cells of dental and with the mesenchymal stem cells of the bone marrow. Periodontal ligament like tissues can be developed from mesenchymal stem cells of dental follicle of third molars in human Mesenchymal progenitor cells that can generate periodontal ligament like tissues and this may be useful for the regenerative periodontal therapy. However, which is the best source of dental mesenchymal cells is still to be determined for successful regenerative therapy. This represents development of a more predictable biologically based therapy for the periodontium.

Identification of periodontal stem cells

Mesenchymal stem cells were first identified in aspirates of adult bone marrow by Friedenstein and colleagues [14] by their capacity to form clonogenic clusters of adherent fibroblastic-like cells or fibroblastic colony-forming units with the potential to undergo extensive proliferation *in vitro* and to differentiate into different stromal cell lineages. Using the above criteria we have recently identified cells that could be classified as mesenchymal stem cells, derived from adult periodontal ligament. The periodontal ligament stem cells exhibited the capacity to generate clonogenic adherent cell colonies when plated under the same growth conditions as described for bone marrow stromal stem cells. Interestingly, the incidence of fibroblastic colony-forming units (aggregates of 50 cells or more) derived from periodontal ligament was greater than that recorded for bone marrow (170 for periodontal ligament stem cells and 14 for bone marrow stromal stem cells per 105 cells plated) [15].

Potential uses of stem cells in periodontal regeneration

In the past for the purpose of repair and regeneration of tissues and organs like spinal chord, heart and bone cartilage, stem cells have been used widely. For the purpose of gene based therapies and tissue engineering both non dental and dental stem cells have been used extensively in the field of dentistry. These technologies have provided a hope and will definitely help in regeneration procedure in periodontal therapy.

Differentiation potential of periodontal ligament stem cells

Many studies have repoted formation of mineralized tissues similar to hydroxyappatite using stromal stem cells of bone marrow, inductive media which contains dexamethasone, ascorbic acid and excess of inorganic phosphate *in vivo* [16]. These findings, suggest that stem cells and periodontal regeneration form mineralized deposits *in vitro* which has been demonstrated for a subpopulation of cells derived from primary explants of periodontal ligament. Periodontal ligament stem cells of humans exhibit a similar capacity to form the Alizarin Red-positive mineralized deposits *in vitro* under the same conditions [17]. The Periodontal ligament stem cells also has multipotential capacity which has been demonstrated by their ability to form Oil-red O-positive lipid-containing clusters of fat cells when they are cultured in the presence of the adipogenic inductive medium. Next, we have to determine the periodontal ligament stem cells capacity to form functional and an organized tissue following implantation *in vivo*. A suitable scaffold is required by these cells like tricalcium phosphate or hydroxyapatite which will help in the formation of the dentin, bone and the cementum *in vivo* [18]. Thus, a typical periodontal ligament or cementum like structure is formed when the periodontal ligament stem cells are placed into a tricalcium phosphate or hydroxyapatite scaffold and then they are implanted subcutaneously into the immune compromised mice [17]. In addition, xenografts of type I collagen-positive periodontal ligament-like tissue formed within the transplants connect with the newly formed cementum which is morphologically similar to the Sharpey's fibers. The cells responsible for the regeneration

of the tissues in the xenografts are identified of human origin which has been identified using human specific anti-mitochondrial antibodies. Also clones of ex vivo expanded periodontal ligament derived fibroblastic colony forming unit have a degree of hetereogeneity in their morphological characteristics, proliferative capacities and differentiation potential. This suggests at various stages of development, there is a mixture of stromal progenitor cells within the total fibroblastic colony-forming unit population and they are maintained by a minor population of multipotential, mesenchymal stem cells with the capacity for self-renewal [19].

Future prospects for stem cells in periodontal regeneration

Stem cells in periodontal regeneration have an excellent future. Now, it is the time to move on to clinical trials. However, it is a new technology and every new technology has more number of questions than answers. There are still many hurdles in moving to clinical trials. Animal studies have shown that mesenchymal stem cells can be used for periodontal regeneration. Various questions arises that what should be the best delivery device, whether to use autologous or allogenic cells, immunogenicity, which tissues will provide the best donor source, cost factor, control of whole. Good manufacturing is the critical need of this technology. Before moving to the human trial, we should know the systematic validation of the specific mesenchymal stem cells as reliable sources for cytotherapeutic use [20].

Potential clinical applications for human derived dental stem cells

Mobilized peripheral blood stem cells have been used as a recognized therapy for hematopoietic bone marrow reconstitution in the cancer patients undergoing myeloablative therapy. The successful results of this therapy has led to investigations of other stem cells like potential novel cellular-based therapies for a number of diseases and congenital defects of the neural tissues, cartilage, bone and muscle tissue and bone marrow stromal stem cells. There are many different types of regenerative cells in the orofacial region, this arises a need of investigation to search appropriate stem cells for regeneration of the orofacial region especially the periodontium. Studies suggest that mesenchymal stem cells and stromal cells of the bone marrow have the capacity of periodontal regeneration. Several studies have been done to test whether periodontal ligament stem cells possess a tissue regenerative capacity similar to that of bone marrow stromal stem cells. Cultured periodontal ligament stem cells of humans have been implanted into surgically created periodontal defects in the nude rats. The results suggest that the periodontal like structure is formed and also periodontal ligament stem cells are attached both to the alveolar bone and the cementum. Recently, in tissue engineering, for regenerating defects created in the periodontal ligament, alveolar bone and cementum selective implanting of autologous bone marrow stromal stem cells and periodontal ligament stem cells in combination with different biocompatible materials⁄ scaffolds using an established ovine preclinical model have been done. More recently, the ovine counterparts of the human bone marrow stromal stem cells and periodontal ligament stem cells have been identified which demonstrates similar functional properties when transplanted into immune compromised mice with the hydroxyapatite⁄ tricalcium phosphate carrier particles.

Challenges in bringing stem cell-based research to practice

Biological challenges

Today we are able to understand the role of progenitor stem cells in healing because of the ability to isolate and characterize stem cells from periodontal tissues. Despite evidences have suggested that regeneration can occur in humans there is still a doubt in successful regeneration especially in cases of advanced periodontal defects. We also have a incomplete understanding about root development and also we know only a little bit about the signalling mechanism. For the periodontal regeneration we must know how to replicate the key cellular events parallel to the periodontal development.

Technical challenges

First, we are not able to provide cell culture environment similar to *in vivo* conditions, which is important to control cell differentiation and proliferation under control. We are also facing technical difficulties in providing appropriate delivery system, cell manipulation and scaffold materials. Furthermore, cell culture medium often has the risk of transmission of infection and is not completely free of pathogenic microbes as they are made up of xenogenic materials like mouse feeder layers and foetal bovine serum. Hence, for human clinical trials we need optimal cell culture conditions which are important to know lineage determination and cell culture. Second, timing is a big hindrance in tissue engineering. Some autologous construct requires weeks to months of ex vivo processing. Although, the processing time can be minimized when using stem cells vs somatic cells, gene mutations and possible karyotyping after culture for long time can also occur. Third, we are in need of an ideal biocompatible scaffold material and its delivery system.

Clinical challenges

Clinical challenges include oncogenic properties of stem cells after their administration to suitable site and functional integration of transplanted tissues into the host. Generally, the expression of major class I and II histocompatibility antigens demonstrate the immunogenicity of human cells and this allows the human body to distinguish foreign cells from its own cells. It is important that we should understand the immunogenicity developed upon transplantation of these stem cells. A low level of class I major histocompatibility antigens is expresses by embryonic stem cells in humans but this expression will be up-regulated with differentiation. However, the production of induced pluripotent stem cells from adult somatic cells is now possible and the differentiation of autologous induced pluripotent stem cells into cell types desired for transplantation is being explored. The use of autologous stem cells for future periodontal regeneration can overcome immune rejection.

Discussion

Regeneration is the best treatment option for a damaged and diseased tooth which will not only maintain the functionality but also the vitality of the tooth. Human teeth can be regenerated using stem cells. Also periodontal

complex and cementum have been regenerated using periodontal stem cells. With the available regeneration options in biotechnology, regeneration of the whole tooth and making it functional is not possible because of the complex structure of the tooth containing enamel, dentin, pulp, cementum and periodontal ligament. We need to further understand the mechanism of interaction among the cells, biomaterials and growth factors to make periodontal regeneration a reality. However, in future we may be able to develop or grow tooth *in vitro* and *in vivo*. Such multipotent stem cells can be obtained from sources mentioned above. After initiation of the tooth germ it can be transplanted into the oral cavity or it can also be grown *in vitro*. It is a difficult process but not impossible. In addition tooth initiation can be started in the oral cavity with the help of growth factors and differentiation factors.

Conclusion

It is the long standing aim of the periodontal therapy to form original form and function of the tissues which are destroyed by the periodontitis. We are in need of ideal regenerative technologies. However, our current regenerative therapies have poor clinical predictability. In order to make ideal periodontal regeneration a reality we must understand periodontal development and the progenitor stem cells. Several studies have reported that stem cells, in conjunction with growth factors and different physical matrices, have the ability to regenerate periodontal tissues *in vivo*. However, there are still numerous technical, biological and clinical hurdles to be overcome. After understanding progenitor stem cells, we may be able to place progenitor cells within a matrix scaffold, together with various signalling molecules in a special and orderly sequence. We must know the underlying processes in periodontal development; mechanisms behind the stem cell differentiation and self-renewal. We still need to study adult stem cells and embryonic stem cells to enhance our knowledge on these cells function and role in disease process. This knowledge will help us provide better treatment modalities and will finally make our dream of gene therapy and stem cell based tissue engineering the real life possibility. Advancement is required both in the clinical setting and delivery of stem cells to make this regeneration of periodontium a reality.

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Conflict of interest

Nil

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