

Stem Cell Mediated Bioroot Regeneration: It's Your Future whether you Know It or Not



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ABSTRACT: Early Tooth loss in humans caused by caries and dental trauma has a serious impact on their oral health-related quality of life. Different approaches such as removable dentures, fixed prosthesis, tooth autotransplantation, allotransplantation & dental implants have been in practice to restore the lost tooth structure, but none of them is completely successful as they present with problems with biocompatibility, inflammatory changes, and failures thus making the problem further more complex. Hence, the development of new methods of tooth replacement has become important. Stem cell-based tissue engineering which can recapitulate the in vivo environment has shown that dental, non-dental, embryonic, and adult stem cells can contribute to teeth formation under favorable circumstances. The concept of 'Bioroot' with supporting evidence that stem cell populations may be present in human teeth provides the opportunity to consider biological tooth replacement. This article enlightens the concept of stem cell-mediated bio root regeneration and their literature evidence and the significance of bio root formation as a potent tooth replacement tool in the future.

KEYWORDS: bio root, biofactors – cell sheets, tooth regeneration, scaffold, stem cells

INTRODUCTION

Tooth loss occurs as a result of various pathological conditions, such as periodontitis, dental caries, trauma, congenital malformations, or age-related changes. In humans, tooth loss can lead to physical and mental complaints that reduce the quality of life. It is associated with global health and economic burden. Removable dentures, fixed partial dentures and metal implants are mainly used to repair these defects; however, they have limitations in terms of provide anatomical structure, tissue regeneration properties etc [1]. Therefore many researchers are working towards the concept of regeneration and exploring new therapeutic strategies for tooth loss.

The tooth root is a multi-structure organ composed of soft tissues of dental pulp, periodontium, and mineralized tissues (dentin and cementum). These mineralized tissues are an integral part of the tooth and play critical roles in maintaining tooth functions and supporting a natural or artificial crown [2,3]. Recent advancements in dental stem cell biotechnology and cell-based tooth regeneration have made it possible to regenerate a living tooth. However, it has been proven impossible to restore an entire tooth, including the crown and root, because of the human tooth structure and the inability of tissue engineering to induce tooth eruption. Alternatively, regenerating the root may be easier than whole tooth regeneration shortly. From the standpoints of anatomy and clinical practice, the tooth root plays a significant role in maintaining tooth function, because it provides a stable anchor for a natural or post-supported crown. So based on the above idea, the concept of Bio-root regeneration through stem cells was proposed in 2006 [1].

The objective of bio-root regeneration is to implant pre-shaped root-like scaffolds combined with stem cells into the alveolar bone to form a functional bio-root, which is capable of supporting post-crown prosthesis (Figure 1). The tissue product that is regenerated should present as a root-like structure with biomechanical properties and elements similar to the natural teeth with histological reproducing a periodontal ligament (PDL) like tissue and a dentin-like matrix structure [4].

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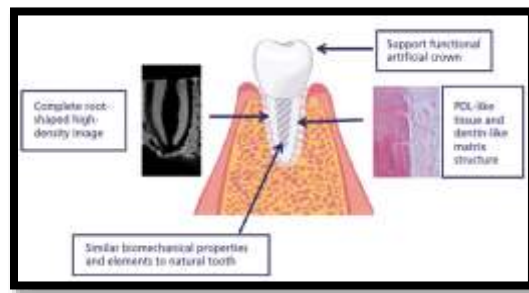


Figure 1: The concepts of functional bio-root regeneration and restoration of tooth loss (Adapted from the Textbook and color atlas of traumatic injuries to the teeth [4])

HISTORICAL PERSPECTIVE OF BIOROOT REGENERATION

Earlier non-biological approaches were used for restoring tooth loss. In 200 AD; an iron stud was used to replace the tooth. In the Mayan Civilization nacre, tooth implants were discovered [5]. Materials such as gold, sapphire, and stainless steel were all used as teeth replacements throughout the generations, Branmark made a breakthrough by introducing one of the most successful and widely accepted titanium implantation systems in 1980 [6]. However, these devices might induce foreign body reactions and run the risk of rejection by the immune system [7]. Therefore, biocompatibility is the need of the hour for the next generation of tooth replacements.

Advances in molecular biology and stem cell technology have resulted in the emergence of regeneration as a new treatment modality in medical science. i.e. "Regenerative medicine". A quarter century after its inception, several treatment procedures which earlier focused on replacement with artificial structures have been substituted with regenerative procedures [8].

As regenerative medicine and tissue engineering technology has developed, researchers are exploring the potential for regenerating living functional teeth. Evidence, that stem cell populations are present in human teeth, provides the opportunity to consider 'new for old' biological tooth replacement [9].

To accomplish tooth regeneration, the natural process of tooth embryonic development can be replicated in vitro or in vivo as an attempt to recreate the nascent growth of tooth germs, and dental stem/progenitor cells. Slavkin et al suggested that cultures of dental pulp cells derived from an early stage of developing dental root and pulp tissue can differentiate into odontoblast-like cells with the capacity to form mineralized nodules in vitro [10]. In 2006, Sonoyama et al showed that stem cell-mediated bio-root regenerated in miniature pig models, similar to that of human teeth in anatomy, development, physiology, pathophysiology, and disease occurrence [11].

MAJOR DOMAINS OF STEM CELL-MEDIATED BIOROOT REGENERATION

There are three major domains of stem cell-mediated bio root regeneration viz stem cells, scaffolds & biofactors - cell sheets.

I. Stem cells

Duality et al in 2006 defined stem cells as "Quiescent cell populations present in low numbers in normal tissue, which exhibit the distinct characteristic of asymmetric cell division, resulting in the formation of two distinct daughter cells, a new progenitor cell and another daughter cell capable of forming a differentiated tissue"[12]. The term stem cell was proposed for scientific use by Russian histologist Alexander Maksimov in 1908 [13].

Two types of stem cells are used in stem cell-mediated bio root regeneration i.e Dental stem cells & Non dental stem cells [4]. They are:

A. Dental stem cells : These comprise of dental pulp stem cells, stem cells from apical papilla, periodontal ligament stem cells & periapical follicle stem cells

B. Non-dental stem cells

A. Dental stem cells:

- 1) Mesenchymal stem cells (MSCs) are multipotent cells that can be differentiated into different types of cells such as osteoblasts, chondrocytes, myocytes, and adipocytes. Since they were first isolated from bone marrow, it was thought that MSCs were located in almost all tissues, perhaps even in neural tissues. Thus, it was speculated that MSCs may be found in adult dental tissues, which also contain a population of multipotential stem cells [14].
- 2) Dental pulp stem cells

The dental pulp contains a population of stem cells which are known as dental pulp stem cells (DPSCs), characterized by the presence of a well-defined layer of aligned odontoblast-like cells expressing the dentin-specific protein DSPP. They are also

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referred to as odontoblastoid cells because these cells appear to synthesize and secrete dentin matrix like the odontoblast cells that they replace [15].

According to a study conclusions given by Gronthos et al, Miura et al in 2003 [4], Dental pulp stem cells can generate dentin/pulp-like structures when mixed with hydroxyapatite/tricalcium phosphate (HA/TCP) ceramic powder and transplanted into the dorsal surface of immunocompromised mice. It has been postulated that perivascular mesenchymal cells surrounding the dental pulp are capable of differentiating into macrophages, fibroblasts, odontoblasts, and osteoblasts following injury [16].

B) Stem cells from apical papilla (SCAP)

SCAP cells are found in the apical papilla located at the apices of developing teeth. It is mainly seen at the junction of the apical papilla and the dental pulp [16].

Because of the apical location, this tissue receives its collateral circulation from the periapical tissue vasculature, which enables it to survive during the process of pulp necrosis. Hence even after endodontic disinfection, SCAP can generate primary odontoblasts, which complete root formation under the influence of the surviving epithelial root sheath of Hertwig [17].

3) Periodontal ligament cells

Human periodontal ligament stem cells have been successfully isolated from the root of extracted teeth. This was first isolated and characterized by Seo et al in 2004. The potential of PDLSCs to develop into other cell lineages and obtain periodontal ligament-like characteristics has been established in vitro as well as in vivo [18]. They possess the ability to differentiate into cementoblast-like cells, adipocytes, and collagen-framing cells along with the capacity to generate a cementum/PDL-like structure [19].

4) Periapical follicle stem cell (dental follicle stem cell)

The dental follicle is a multipotent tissue as it can generate cementum, bone, and pdl from the ectomesenchyme fibrous tissue. These cells were first isolated from the follicle of impacted third molars. In 2002, Handa K isolated progenitor cells from bovine dental follicles & showed that these cells can differentiate into mesenchymal-derived cells like cementoblasts, adipocytes, and chondrocytes [17, 18].

B. Non-dental stem cells:

Non-dental stem cells such as embryonic stem cells, neural stem cells, and adult bone marrow-derived cells have the potential of expressing odontogenic genes. One of the studies has shown that recombination between non-dental stem cell aggregations and embryonic oral epithelium transplanted into adult mice renal capsules resulted in the development of tooth structures and associated bone [20].

II. Scaffolds

Scaffolds can be defined as biocompatible structures that support cell growth and provide a suitable environment for tissue formation. The scaffold is also an essential element in tissue engineering. It is still unclear exactly what shape and size a bioengineered tooth root should be, to produce the best retention and function [21]. This is important since bioengineered organ/tissue shapes and sizes are routinely controlled by scaffold fabrication. Therefore, exploring and optimizing the design of scaffolds has great significance for further research and clinical utilization of bio-root regeneration [22].

A. Spherical polyglycolide/poly-L-lactide (PGA-PLLA) scaffolds:

PGA-PLLA scaffolds are frequently used and have good biocompatibility and cell-retaining behavior. PGA-PLLA has been used as an artificial scaffold for cell transplantation and degrades as the cells excrete extracellular matrix [22]. They were used as a scaffold to demonstrate that dentin-like tissue could be formed and pulp-like tissue could be regenerated after 3–4 months. PGA-PLLA in a 50:50 mixture has a degradation time of about 8 weeks [23].

B. Hydroxyapatite/TriCalcium Phosphate (ha/TCP) scaffolds:

Researchers have shown that human bones are generated after xenogeneic transplantation of bone marrow stromal cells with HA/TCP as a carrier vehicle. In one of the researches, consistent bone formation by human MSCs was achieved within HA/TCP ceramics in the form of blocks, powder, and HA/TCP powder type I bovine fibrillar collagen strips; and bone was maintained for at least 19 weeks [24].

C. Treated dentin matrix (TDM) scaffolds:

Treated dentin matrix scaffold, when seeded with dental pulp stem cells, and transplanted to the alveolar bone of swine, has shown stable histological regeneration and also improved masticatory function. Krebsbach et al conducted studies on evaluating bone formation in vivo by comparison of osteogenesis in transplanted mouse and human marrow stromal fibroblasts validated the use of TDM scaffold 9.4mm in length and 4.9/3.4mm in diameter for effective biological tooth regeneration [25].

III. Biofactors –cell sheets:

Cell sheet engineering has been used as an alternative approach to tissue engineering in periodontal tissues. Biofactors such as continuous cell sheets can enable the preservation of cellular junctions, endogenous extracellular matrix (ECM) and mimic cellular microenvironments in terms of various mechanical, chemical and biological properties [26]. At present, several improvements

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have been made to harvest the living cell sheet more easily. In some studies, dexamethasone and ascorbic acid phosphate (vitamin C) were used to create cell sheets to enhance bone formation [27].

REGENERATION METHOD OF STEM CELL MEDIATED BIROOT

In 1949, the Hormel Institute of the USA developed a Minnesota miniature pig and used it as a large animal model in medical studies for scientific, economic, and ethical reasons [28]. The oral maxillofacial region of miniature pigs is similar to that of humans in development, anatomy, physiology, pathophysiology, and disease occurrence. This miniature pig had both deciduous & permanent dentition, thus helping to evaluate the initiation of tooth formation. Various studies were done in pig models which elaborated that stem cells isolated from cultures of pigs are similar to human stem cells [29].

Method of stem cell-mediated bio root regeneration [4] included the following steps as illustrated in figure 2:

- I. Isolation of autologous or allogenic stem cells in culture for growth.
- II. Scaffold fabrication which provided the suitable root shape and acted as a membrane containing an inner post channel space to allow the subsequent installation of a porcelain crown.
- III. Stem cells seeded on the scaffold and harvested stem cell sheet.
- IV. Preparation of the bio-root complex before implantation.
- V. Preparation of implantation sockets.
- VI. Implantation of bio root complex.
- VII. Regeneration of bio root.

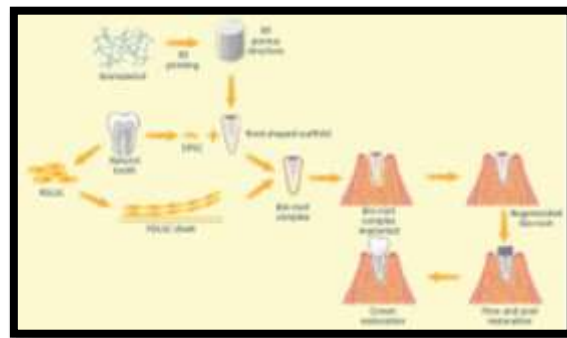


Figure 2: Diagram showing the use of bio-roots in the treatment of tooth loss. Dpsc (dental pulp stem cells); pdpsc (periodontal ligament stem cells). (Adapted from Textbook and color atlas of traumatic injuries to the teeth [4])

THE CLINICAL CRITERIA FOR THE SUCCESS OF STEM CELL-MEDIATED BIO ROOT REGENERATION ARE

- I. To have a root-like structure with computed tomography (CT) examination showing a complete root-shaped high-density image.
- II. To have similar biomechanical properties and elements to natural teeth.
- III. To have histology showing periodontal ligaments (PDL) like tissue and a dentin-like matrix structure [30, 31].

PROSPECTS OF STEM CELL-MEDIATED BIROOT REGENERATION

- I. The biomechanical properties of the bio-root such as compressive strength, elasticity modulus, and torsional force are very similar to those of the natural tooth root.
- II. Bioengineered dentin results in inducing cementogenesis and PDL formation.
- III. Element analysis has shown that the bio-root has similar element content (mainly calcium, phosphorus, and magnesium) to that of natural teeth, indicating that biological changes do occur during the bio-root regeneration.
- IV. Stem cell-mediated root regeneration provides opportunities to regenerate bio-root and its associated periodontal tissues, which are necessary to maintain the physiologic functions of teeth [4, 32].

LIMITATIONS OF STEM CELL-MEDIATED BIROOT REGENERATION:

The sources of dental stem cells are potentially limited and their activity is hard to maintain after implantation. Also, the lack of an effective biological scaffold fabrication is another limiting factor. The procedure lacks precise control of directional differentiation of dental stem cells as well [32].

CONCLUSION:

Findings of in vivo studies done on animal models have shown that the stem cell-mediated bio-root tooth regeneration approach can functionally generate tooth tissue and also has benefits over conventional dental

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implants. Nevertheless, results of the ongoing preclinical and clinical trials will be required to assess the therapeutic potential of bioroot in clinical applications for tooth regeneration.

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