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'Bioroot' –A Nascent Paradigm In Tooth Regeneration

Ponnudurai Arangannal, Jeevarathan J, Amudha S , AarthiJ, Vijayakumar M

Department of Pedodontics & Preventive Dentistry

Sree Balaji Dental College & Hospital'

Narayanapuram, Pallikaranai,

Chennai 600100

drapdurai66@gmail.com

ABSTRACT

Early Tooth loss in children caused by caries and dental trauma has a serious impact on their Oral health related quality of life .From the past to till date various approaches such as removable dentures , fixed prosthesis have been into practice to restore the lost tooth structure. Tooth auto transplantation, allotransplantation and dental implants have existed for many years, but not 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 desirable and is a realistic possibility in children of growing age .Stem cell-based tissue engineering which can recapitulate the in vivo environment showed that dental, non-dental, embryonic and adult stem cells can contribute to teeth formation under favourable 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 'new for old'. This article enlightens on the various materials used in tooth regeneration, their literature evidences and the significance of bioroot formation as potent tooth replacement tool in the future.

Keywords: tissue engineering, stem cells , tooth regeneration/transplantation

INTRODUCTION :

Regeneration of a functional and living tooth structure could be a very remarkable therapeutic intervention in replacing a tooth. Liu et al and Cao et al 2010;Rimondini et al and Mele et al 2009 quoted that regeneration of the oral structures such as the periodontium, alveolar bone, dentine pulp complex, craniofacial bone, mucosal tissue, tongue muscle, and for returning the function of salivary glands have been existing through these years.[2]. Ikada et al in 2009 ,though it is very complicated to regenerate the entire tooth including the crown , the regeneration of the pulp dentin complex valuably could support a prosthesis The concept of a 'bio-root', which is generated through stem cell-based tissue engineering, was proposed in 2006 (1). The idea behind is to implant preshaped root-like scaffolds combined with stem cells into the alveolar bone to form a functional bio-root, which is capable of supporting post crown prostheses. The tissue product that is regenerated should present as a root like structure with biomechanical properties and elements similar to the natural teeth with histologically reproducing a periodontal ligament (PDL) like tissue and a dentin-like matrix structure.

TOOTH REPLACEMENT IN THE PAST –NON BIOLOGICAL APPROACHES:

Non-biological approaches have been used for restoring lost tooth for centuries. The early history of replacing a human tooth with an iron stud can be traced back to 200 AD [2], and nacre tooth implants have been discovered from the Mayan civilisation [3]. Materials such as gold, sapphire and stainless steel were all used as teeth replacements throughout the generations. In the 1980s, Branemark made a breakthrough by introducing one of the most successful and widely accepted titanium implantation systems. However, these devices all might induce foreign body reactions and run the risk of rejection by the immune system, and thus biocompatibility is the need of the hour for next generation of tooth replacements.[4]

NEW ARENA IN TOOTH REPLACEMENT – BIMIMETIC APPROACHES :

Biomimetic restorations have now come into daily practice for restoring partial teeth structures, such as dentin and periodontal ligament (PDL). The best example for dentinogenesis is the commonly used calcium hydroxide, which is used as a pulp-capping agent to induce dentin bridge formation [4,5]. Guided tissue regeneration methods have been successfully used to regenerate periodontal tissues by using a barrier membrane to prevent gingival epithelium and connective tissue growing to the periodontal space, thus allowing functional PDL regeneration [6]. However tooth development is a complex process involving redundant and reiterative signals with temporal and spatial protein expression patterns.[28] Tissue engineering dimension has opened up more than a decade ago, whereby living cells could be used to develop biological substitutes for tissue replacements. With the advance of material science and knowledge of biological processes, tissue engineering has become an alternative approach for restoring tissues and even organs instead of autografts, allografts and artificial prostheses [9-11]. Whole tooth regeneration using tissue engineering principles is progressing [12]. In order to accomplish tooth regeneration, the natural process of teeth embryonic development can be replicated *in vitro* or *in vivo*. as attempt to recreate nascent growth of tooth germs, dental stem/progenitor cells . Ability to reproduce the human tooth like structure from tooth tissues of rats and humans suggests that there is potential for the regeneration of mammalian dental tissues. Various precursors of specialized tooth-associated cell types have been identified, including their characteristics and properties in the postnatal organism (12,13). 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* (12). Researchers Duailibi MT DS et al , Young CS et al , Bartlett JD et al have found tooth tissue in rats by using cultured rat tooth bud cells (14). Sonoyoma et al in 2006 ,showed that stem cell-mediated bio-root has been regenerated in miniature pig models, similar to that of human teeth in anatomy, development, physiology, pathophysiology and disease occurrence (1).

COMPONENTS OF STEM CELL MEDIATED 'BIOROOT' TOOTH REGENERATION :

1. STEM CELLS:

Mesenchymal stem cells (MSCs) are multipotent cells that can differentiate into different types of cells such as osteoblasts, chondrocytes, myocytes and adipocytes. The MSC s are exclusively present as groups of multipotent stem cells in various dental tissues which are classified as dental stem cells and the rest as non dental stem cells.[26]

DENTAL STEM CELLS:

- The **DPSC(Dental Pulp Stem Cells)**which regenerate dentin / pulp like tissues, transplants are characterized by the presence of a well-defined layer of aligned odontoblast-like cells expressing the dentin-specific protein DSPP. The collagen matrix mimics the structure of primary dentin with ordered fibres present perpendicular to the odontoblast layer (12)

Recently, dental pulp stem cells (DPSCs) were identified from both adult human dental pulp [12] and human exfoliated deciduous teeth , (SHED) stem cells which possess high proliferative and colony-forming ability. According to study conclusions given by Gronthos et al ,Miura et al in 2003 ,the individual colonies show comparable levels of heterogeneity in growth and developmental capacity, can generate dentin-like structures[16] and survive in the brain with neural marker expression when implanted into immune compromised mice [15].It has been postulated that perivascular mesenchymal cells surrounding the dental pulp are capable of differentiating to macrophages, fibroblasts, odontoblasts and osteoblasts following injury [12].

- **STEM CELLS FROM THE APICAL PAPILLA :(SCAP)**

SCAP arise from epithelial stem cell niche located at their apical ends known as the cervical loop, which is the junction of the inner enamel epithelium and the outer enamel epithelium at the apical end of the enamel organ, composed of a core of stellate reticulum cells and surrounding basal epithelial cells contacting the dental mesenchyme. The cervical loop is considered to be an important region in odontogenesis because it exists more primitive (less differentiated) group of cells [19]. Slavkin *et al.* showed that recombination of epithelium and the dental papilla mesenchyme from rabbits continuously growing incisor cervical loop regions could recreate a functional tooth germ on the chick chorio-allantoic membrane. [19]SCAP, isolated from the external apical root foramina of extracted 3^{RD} molars, were capable of differentiating into odontoblasts/osteoblasts and other cell such as adipocytes, which were similar to DPSCs and bone marrow mesenchymal stem cells (BMMSCs) which would regenerate typically a dentin structure (1).

- **PERIODONTAL LIGAMENT STEM CELLS :**

PDLSC cells constitute clonogenic cluster of fibroblast like cells , capable of differentiation into PDL-like structures, bone and cementum [20]. Studies done on mice mimicked the formation of collagen ,physiological attachment of sharpeys fibres similar to those present in pdl .[13]

- **PERIAPICAL FOLLICULAR STEM CELLS:**

Periapical follicle stem cells (PAFSCs), isolated from the apical end of developing roots of human third molars, showed stem cell properties and a higher proliferation rate *in vitro*. Meanwhile, PAFSCs also showed the regenerative capability that is needed to produce similar cementum/PDL-like complex *in vivo*. (21).

NON DENTAL STEM CELLS :

Non-dental stem cells such as embryonic stem cells, neural stem cells and adult bone marrow-derived cells all have the potential of expressing odontogenic genes. 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.[26]

2. SCAFFOLD:

The scaffold is an essential element in tissue engineering. The size and shape of the scaffold should be suited to produce best retention and function of the bio engineered tooth roots. Various materials available to be used as scaffold in tissue engineering are as follows:

Spherical polyglycolide/poly-l-lactide (PGA-PLLA) scaffolds :PGA-PLLA scaffolds are frequently used and have fantastic biocompatibility and cell-retaining behaviour. scaffolds are fabricated through bonding PGA felt and PLLA in chloroform in a spherical polyvinylsiloxane negative mould (22).

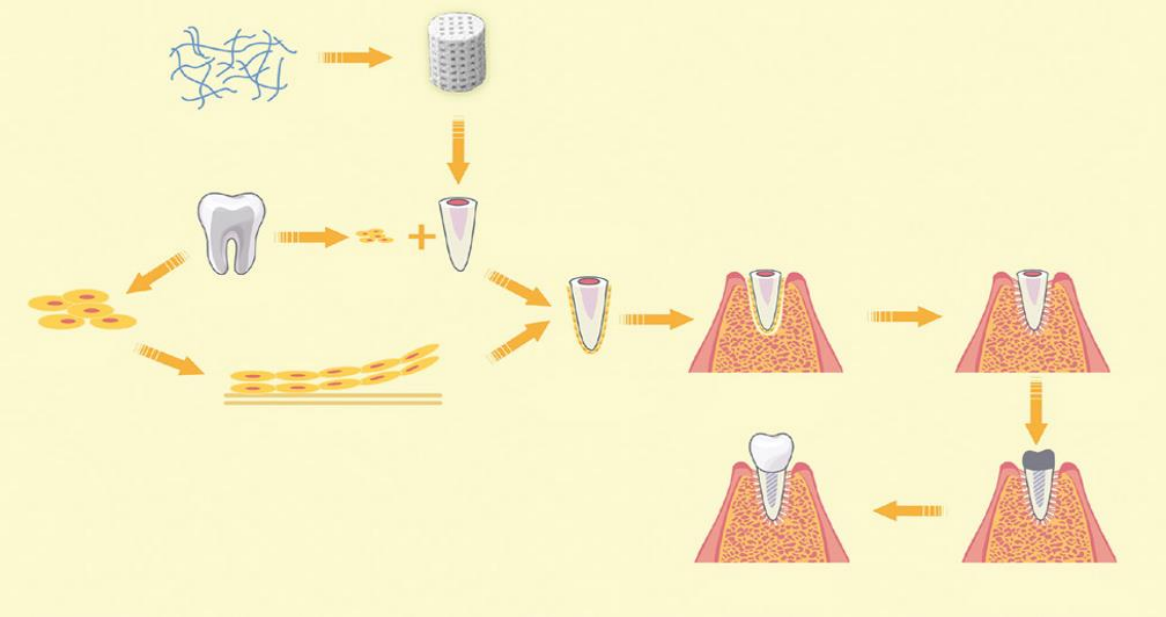
Hydroxyapatite/tricalcium phosphate (HA/TCP) scaffolds : xenogeneic transplantation resulted in Consistent bone formation by human MSCs using HA/TCP ceramics in the form of blocks, powder as a carrier vehicle along with type I bovine fibrillar collagen strips; and bone was maintained for at least 19 weeks (23).

Treated dentin matrix (TDM) scaffold
Treated dentin matrix scaffold when seeded with dental pulp stem cells,transplanted to alveolar bone for swine have 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 [24]

3. BIO FACTORS –CELL SHEETS:
Biofactors such as continuous cell sheets can enable the preservation of cellular junctions, endogenous extracellular matrix (ECM) and mimicking cellular microenvironments in terms of mechanical, chemical and biologic properties.. Cell sheet engineering has been developed as an alternative approach to tissue engineering in periodontal tissues (29).

REGENERATION PROCEDURE OF STEM CELL MEDIATED BIO ROOTS :

- **MINIATURE PIG MODELS:** The Minnesota miniature pig models have been used in study of bioroot formation due to remarkable similarities with the human developmental anatomy and pathophysiology and have been used as a large animal model in medical studies for scientific, economic and ethical reasons (30).It also presents with both deciduous and permanent dentition , thus enabling us to evaluate the initiation of tooth formation .Also it has been suggested that , stem cells isolated from cultures of these are similar in nature to the human stem cells.
- Isolation of autologous or allogenic stem cells in culture for growth .Scaffold provides the suitable root shape and acts as membrane containing an inner post channel space to allow the subsequent installation of a porcelain crown. Stem cells are seeded on to the scaffold and harvested .the extracted socket that is prepared surgically before implantation .The scaffold containing the stem cells is then inserted to the site and let to heal over a period of time .On re opening after implantation , porcelin crowns matching the swine models fabricated and inserted . CT and histological examinations are performed at regular interval after 6 month period to evaluate the bio root formation .[27]



Schematic diagram for the use of bio-roots in the treatment of tooth loss. DPSC, dental pulp stem cells; PDLSC, periodontal ligament stem cells. *Adapted from adreasens text book of traumatic injuries to the teeth 5Th edition [26]*

PROSPECTS OF BIOROOT TOOTH REGENERATION

Biomechanical properties such as compressive strength , torsional force and modulus of elasticity of the bio root regenerated were close to that of the normal tooth roots [2]. Element analysis showed that the bio-root had similar element content (mainly calcium, phosphorus and magnesium) to that of natural teeth, indicating that biologic changes had occurred during the bio-root regeneration. Bioengineered dentin is able to induce cementogenesis and PDL formation, and condense PDL arranged perpendicularly on the dentin surface via a layer of cementum-like tissue. The results indicated that biologically tissue engineered dentin could be reproduced using an inductive substrate and could be used as a further as medium for cementum and PDL tissue engineering (27). Stem cell-mediated root regeneration provides opportunities to regenerate bio-root and its associated periodontal tissues, which are necessary for maintaining the physiologic function of teeth. All recent studies indicate that a regenerative approach is feasible using allogeneic MSCs to regenerate bio-roots for tooth loss in large animals (e.g. the miniature pig).[26]Thus this new dimension in reinstating a lost tooth can outweigh the drawbacks associated with pedo implants when given in a growing child.

PDLSC

LIMITATIONS OF BIO ROOT TOOTH REGENERATION :

Crown restoration

Although the data from studies promise in the successful regeneration of a dental stem cell–mediated bio-root, much more clinical evidences performed in human trials are need to ensure the potential of this procedure .The maintenance of multipotent MSCs *in vitro*,the sources of dental stem cells are potentially limited and their activity is hard to maintain after implantation and lacks precise control of directional differentiation of dental stem cells . Another problem is there is lack of an effective biological scaffold till date, there is no specific scaffold

for bio-root regeneration. In addition to the problems associated with tissue engineering, there are many factors that affect and restrict the regeneration process in vitro and in vivo (Daley and Scadden 2008). [31]With improvements in the scaffold, stem cell quality control, and surgical operation, it is possible that the bio-root will be used in clinical applications in the future.[1]

CONCLUSION :

In conclusion, findings of in vivo studies done in animal models showed that bioroot tooth regeneration approach can functionally reinstate a tooth tissue also had potential advantages over conventional dental implants in growing children. However results of the on going preclinical and clinical trials will be needed to assess the therapeutic potential of bio roots in clinical applications for teeth regeneration.

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