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# A New Paradigm of Tooth Replacement: Biotooth

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## Abstract

Tooth from the oral cavity could be lost or extracted due to trauma, dental caries, periodontal diseases and ageing. It affects the aesthetics, mastication, speech as well as the psychological health of the people. There are various aspects to replace the missing tooth such as RPD, FPD or dental implants. Dental implants are one of the most common replacement choices but it also has certain limitations such as functionality and longevity along with compromised physiology and plasticity with respect to the natural tooth. Periodontal ligament has a fundamental role in cushioning high mechanical load but unfortunately, it is absent around dental implants. As a result, dental implants have shorter lifespan than natural teeth and increases susceptibility to infections. Stem cell-based tissue engineering encourages the formation of biological tooth (Bio Tooth, i.e., living tooth). It mimics all the events which occur in the initiation of odontogenesis, having all anatomical parts as that of teeth and functions similar to natural teeth showcasing regenerative capacity, eruption of new teeth and response to injury.

## Keywords

Stem Cells, Bio Tooth, Biological tooth, Biodontics, Bionic, Tissue Engineering, Tooth replacement, Regeneration.

## Introduction

Loss of teeth is the most commonly occurring diseases in the patients having age above 60 years, affecting many physiologic processes in their lives namely, aesthetics, masticatory sufficiency and their quality of life. The treatment with the dental implants is the current usual procedure which dominantly focuses on the mechanical solutions to the tooth loss and have a huge success rate and popularity. However, despite their long history, there are a number of limitations in functionality and longevity of the implants. Dental implants cannot be the ideal solution for the

replacement of the teeth since the physiology and plasticity of the naturally formed teeth is not respected. The conventional implant attachment does not include the periodontal ligament (PDL), which modulates the mechanical stress during mastication and occupied the space between the tooth root and alveolar bone. PDL is also a prominent region for proprioception and contributes to the collective function under the control of the central nervous system. The absence of PDL makes the bone tissue vulnerable when excessive forces are applied during mastication. As a result, implants have shorter life span and more susceptibility to infections[1].

The main concept in tooth replacement is the possibility of creating functional biocompatible type of structure and efforts are being made to regenerate and mimic the entire tooth. The main aim is to develop fully functioning tooth-like structure in the place of missing tooth either in-vivo or in vitro using stem cells. Bio-tooth is the biologically generated tooth that is precisely regenerated and re-integrated in the jaw of the patient after tooth loss. It is a genetically engineered tooth created from the stem cells.The stem cells for a bio tooth are obtained from primary tooth, unerupted tooth bud, third molar, chord cells (blood) and tissue engineered cells (adipose tissue, hair follicle). These cells undergo mitotic cell division and differentiate into specialized cell types[1].

Several factors have to be looked before making a bio tooth. The cells should be easily isolated from the oldindividuals, mostly suffering from tooth loss. These cells can be easily expanded in vitro to yield enough cell populations necessary for tooth reconstruction. Then, the odontogenic micro-environment must be found that can facilitate these cells to form a three- dimensional bio-tooth in vitro or in vivo. The newly formed bio-tooth made from these cells should have the capacity to continue its development, generate functional root-periodontal complex, and perform directional eruption at the right place in the environment of adult jaws[2]. Finally, the size and shape of these bio-teeth must be controllable in order to match the patient's own teeth and reconstruct normal occlusion. Therefore, this bio-tooth must be similar to the basics of tooth growth and development. Stem cells which help in the generation of the bio-tooth have a unique property of developing into many types of cells throughout the life on simulation[1].

## Stem Cells

Stem cells play an important position in tissue and organ repair becauseof its incredible properties of self-renewal, differentiation and rise of specialized cells from unspecialized cells. The concept of regenerative capability of stem cells is solely carried out in periodontics due to the fact that any form of periodontal disease is the most common cause of alveolar bone loss and tooth loss which will thereby restrict the property of dental implant to restore and repair the function of the natural missing tooth.

Regenerative periodontal/bone therapy was primarily based totally on the usage of scaffolds. In the first generation of this technology, osteoconductive membranes and bone graft materials have been used as a framework for cells to migrate into the periodontal tissue to permit it to regenerate at its normal healing rate. The second-generation technology applied as osteoinductive materials, which includes growth factors, to stimulate periodontal tissues to flourish at an increased rate. Treatment protocols primarily based on these ideas which have already been extensively infiltrated standard dental practice due to the fact that they make up only non-viable materials during the

surgical process and are consequently applied without any difficulty. Mesenchymal /stromal stem cells (MSCs)primarily based regenerative therapies have been established as a third-generation technology for regenerative periodontal/bone therapy mainly in clinical research and scientific studies facilities which include college, university and hospitals. Cell construction technologies, which include cell sheets, have currently been brought to regenerative dentistry as a fourth-generation technology, and scientific trials are now under way. Future fifth-generation technology are predicted to use oral tissue-derived induced pluripotent stem (iPS) cells and genetically modified stem cells to create an extra physiologically analogous substitute for tissue/organs, which includes bioengineered periodontal tissues/teeth[4].

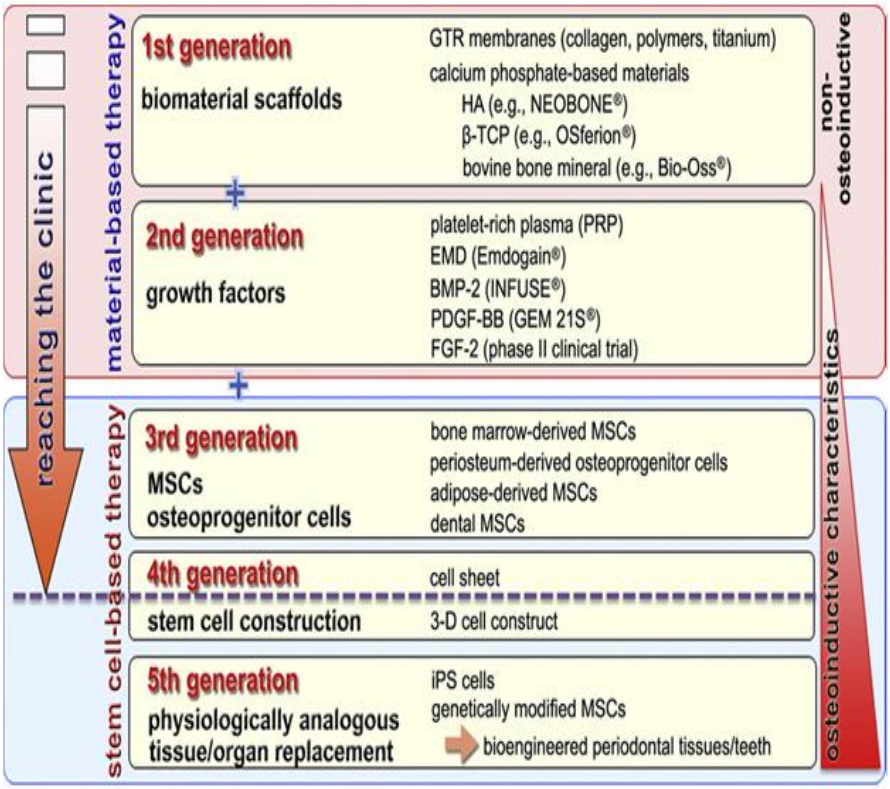



***Figure 1:*** *Progress in regenerative periodontal/bone therapies. Regenerative periodontal/bone therapies are broadly categorized as material-based therapies (first generation biomaterial scaffold-based approach and second-generation growth-factor-based approach) and stem-cell-based therapies (third-generation MSC/ osteoprogenitor cell-based approach, fourth-generation stem-cell construction-based approach, and fifth-generation physiologically analogous tissue/organ replacement approach). Technologies from the first to the fourth generation have already reached the clinic[4].*

On the basis of the site of origin, stem cells are categorized as:

1. Embryonic stem cells (ESCs) or post-natal stem cells
2. Somatic stem cells or adult stem cells
   - Hematopoietic stem cell
   - Mesenchymal stem cell
3. Induced pluripotent stem cell

ESCs are derived the best from 2 to 11- day old embryo, referred to as blastocyst. They are totipotent

cells sustained in an undifferentiated state for the lifetime and so are considered immortal[1]. Despite having variety of benefits, if stem cells are eliminated from the embryo, it will certainly destroy the embryo itself, making this as a critical shortcoming of ESCs. Therefore, it is only confined to researches, disease modelling, drug screening.

Adult stem cells are multipotent cells, which means they are able to differentiate into a couple of cell type but not all cell types. They have a unique property of plasticity, i.e., having the ability to expand beyond their recognized potential irrespective of the parent cell from which they are derived[1,2]. Adult stem cells can be hemopoietic stem cells or mesenchymal stem cells (MSCs).

Induced pluripotent stem cells (iPS) are pluripotent cells artificially generated through genetic manipulation of somatic cells. iPS cells may be generated from absolutely differentiated non-pluripotent cells and possess pluripotency similar to that to ESCs.

iPS cells and ESCs are comparable in terms of expression of certain stem cell genes and proteins, doubling time, chromatin methylation patterns, embryoid body formation, teratoma formation, viable chimera formation, potency, and differentiability. Like ESCs, iPS cells have potential for proliferation and differentiate into all derivatives of the three primary germ layers (ectoderm, endoderm and mesoderm) and many mature in vitro. They have the property of self-renewal if cultured under the same situations as of ESCs. They are also capable of differentiation into mature osteoblasts and produce hydroxyapatite having crystal structure similar to that MSC-associated hydroxyapatite[3].

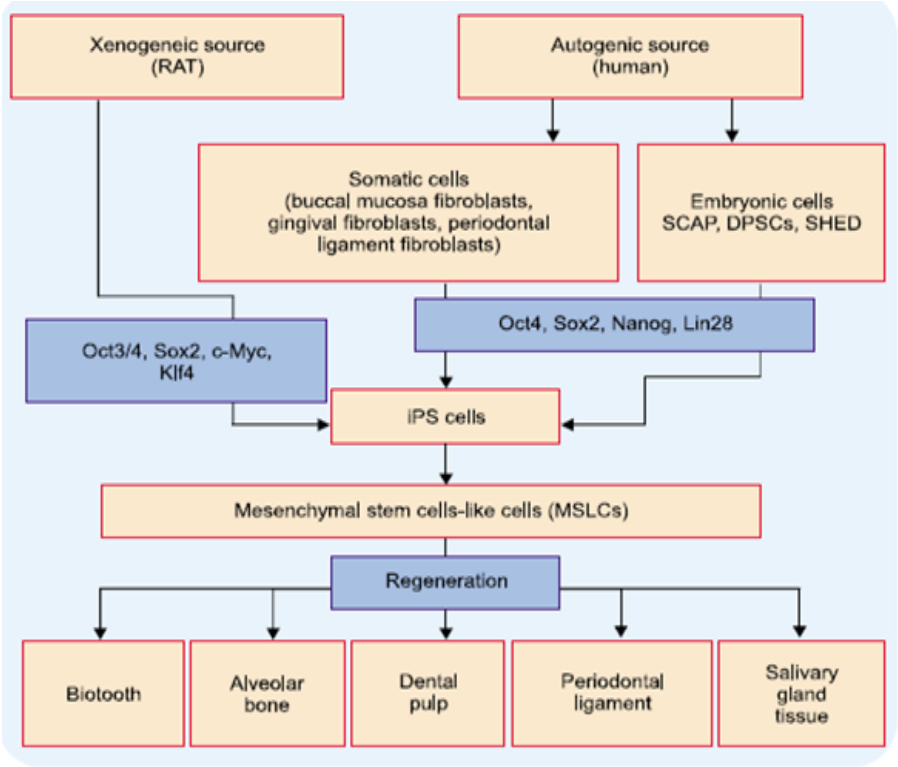



***Figure 2:*** *sources, generation and applications of iPS cells in dentistry*[3].

## Sources of Stem Cells

iPS can be derived from stem cells in apical papilla (SCAP), dental pulp (DPSCs) and primary teeth (SHED), third molars, buccal mucosa fibroblasts, gingival fibroblasts and periodontal ligament fibroblasts[3].

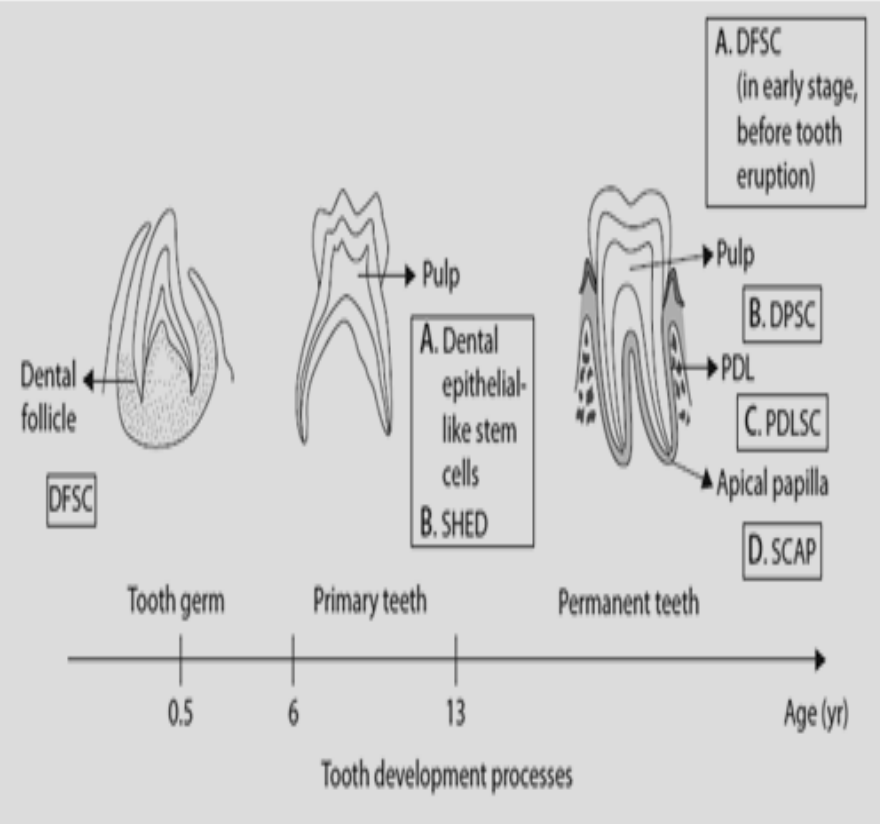



***Figure 3:*** *Tooth developmental stages and the derivation of dental derived stem cells. DFSC = dental follicle stem cells; SHED = stem cells from human primary exfoliated deciduous teeth; DPSC = dental pulp stem cells; PDLSC = periodontal ligament stem cells; SCAP = stem cells from apical papilla*[5].

## SCAP (Stem Cells in Apical Papilla)

It is derived from the tissue developing at the apex of the root, known as apical papilla. Yan et al., studied the accessibility and feasibility to generate iPS cells from SHED, SCAP and DPS cells. It was observed that all 3 cells can be reprogrammed into iPS cells at a higher rate than fibroblasts. They formed embryoid cells in vitro and teratomas in vivo containing tissues of all 3 germ layers and can be used as an alternate source of iPS cells

## DPSCs (Dental Pulp Stem Cells)

They have better tendency than dermal fibroblasts in the production of iPS cells.

## Stem Cells from Primary Teeth

SHED and immature DPSCs have higher regenerative potential than skin fibroblasts. A study by Toriumi et al., concluded that cells competent for iPS generation are more in number in root cells than crown cells of primary teeth and are a more potent alternative.

## Stem Cells from Third Molars

MSCs from third molars have the tendency to generate iPS cells by retroviral transduction without

using the transcription factors responsible for carcinogenesis (i.e., c-Myc). Although, third molars are discarded usually, but they provide valuable, viable and economical source for the generation of iPS cells.

**Oral Mucosa**

They are a good source of iPS cells because of their simple and safe retrieval process with no functional or aesthetic damage and rapid wound healing.

**Gingival Fibroblast Cells**

Itis easily obtained from the oral cavity and have better immunomodulatory properties as compared with the other tissue derived stem cells.

**Periodontal Ligament Fibroblasts**

MSCs-like cells generated from the PDL- iPS cells have a superior capacity to form physiological bone and connective tissue, both in vivo and in vivo as compared to MSCs derived from gingival and lung fibroblast.

**Dental Stem Cell Banking**

A recent animal study established that human dental-pulp-derived stem cells might additionallyoffer more therapeutic benefit for treating spinal cord injury. However, the use of a patient's own dental-tissue-derived stem cells at the time of therapeutic necessity has anextreme limitation due to the fact that it would require the extraction of a remaining tooth. Dental stem cell banking[4], i.e., the method of storing stem cells received from patients' deciduous teeth and wisdom teeth, can be one of the method to recognize the potential of dental-stem-cell-based regenerative therapy. Recently, cell/tissue banks in the dental field have beenplanned and placed into practice in numerous countries, e.g., Advanced Center for Tissue Engineering Ltd., Tokyo, Japan; Teeth Bank Co., Ltd., Hiroshima, Japan; Store-A-ToothTM, Lexington, USA; BioEDEN,Austin, USA and Stemade Biotech Pvt. Ltd., Mumbai, India. Oncestem-cell-containing tissues, which includes PDL, pulp tissues, apicalpapilla, or the tooth itself, are received from the patient, theymay be cryopreserved for decades to preserve their regenerativepotential[6,7,8]. Dental stem cells may be isolated fromthe cryopreserved tissue/tooth whenever required for future regenerative therapies[7,8]. These autologous stem cells given to a patient might be diagnosed as host cells and must consequently be tolerated by the immune system. Stem-cell-based tissue engineering therapies using stem cell banking have now no longer but been bereported. Therefore, the utility of stem cell banking in dentistry must be cautiouslyevaluated. In addition, legislation for the banking system is important as it provides bio-insurance for a future use that is highly unlikely. Checks and audits need to be carried out to decide whether or not the banking organization can function well into the future, and whether or not the cryopreserved cells and tissues are maintained in desirable quality for future use in transplantation.

**Strategy and Need of Tooth Replacement with Biological Method**

Replacement of missingteeth presently include fixed or removable prostheses or dental implants. The use of dental implants is the most rapidly growing area of dentistry, currently increasing by 15–20% per year. Dental implants contain drilling a hole into the jawbone into which a titanium rod is screwed that is capped through a plastic or ceramic 'tooth' crown. Despitebeing the current 'state-of-the-art' in tooth replacement, the technology on which implants are based has beenaround for thousands of years[1]. Thus, comes the need of tooth replacement with some biological process and leads to the birth of Bio-tooth.

The factors which play a critical function for tissue engineering are[1,10]:

1. **Morphogenic Signals:** Growth factors and differentiation factors play a crucialfunction in multiplication and differentiation of stem cells. Bone morphogenic proteins (BMPs), which can be the multifunctional growth factors, belong to the transforming growth factor beta (TGF-β) super family and cytokines of the immune system play a vital role in organogenesis, for example, in differentiation of dental pulp stem cells (DPSCs) into odontoblasts, which is the primary requirement of tooth tissue engineering.
2. **Responding stem cells:** They are initially attained from the patient and preserved under proper situation to uphold their distinctive capability to differentiate into a wide-ranging cells, are later coaxed within side the laboratory to convert it into a tooth bud.
3. **Scaffold:** It offers a mechanical support to the cells required for regeneration of any tissue and it must be biodegradable and speed of degradation has to coincide with the speed of tissue development. The scaffold must be permeable, which aids in cell nutrition, proliferation, and migration for tissue vascularization as well as formation of new tissues. Mechanical stability of the implant is progressed through the porous surface by the mechanical interlocking among the scaffolds and surrounding tissues.

There are two main approaches in constructing a new whole tooth. The first implies the *in vivo* implantation of tooth germ cells that have been previously generated from numerous populations of stem cells or dental progenitor cells and grown *in vitro* for some time. Organotypic culture is the most appropriate of the techniques for the development of the teeth *in vitro*[10, 11].

The different technique includes implanting into the jaw tooth-shaped polymer scaffolds which can be packed with *in vitro* expanded stem cells or dental progenitor cell populations. Ideally, this implant has to reproduce the 3D structure required for the transplanted cells to support their differentiation and avoid xenograft rejection[10,11,12,13].

## Biotooth as Third Dentition

Bio-tooth is better than dental implants and involves mimicking and reconstruction of the whole tooth in the oral cavity. The problem encountered here is the right shape of the tooth which involves four basic ways[1,2,9]:

1. **Reconstruct the mature tooth as it appears in the oral cavity**: The concept of construction of adult tooth has recently been proposed by Pamela Robey and colleagues in 2005[14]. All the components of the tooth, namely, enamel, root, crown and dental pulp are reconstructed separately from the cells and different materials.Teeth require anchorage, bone marrow stromal cells (BMSCs) and hydroxyapatite/ tricalcium phosphate (HA/TCP) could be used to engineer the alveolar bone. The dental pulp and enamel could be constructed using dental pulp cells and HA/TCP in an enamel-like crown mould, whilst the periodontal ligament attaching the tooth to bone could be obtained from periodontal ligament stem cells (PDLSCs)[1].

   **Advantages**: High level of control on the process and the possible automation and scale-up. This procedure is highly technique sensitive thus causing more technical difficulty.

2. **Inducing a Third Dentition**: In this step, there is an addition of molecules from either of the two previously present dentitions i.e., primary and succedaneous dentition in the development of initiating the de novo of the tooth post loss of the tooth[2,9].
3. **Create a Tooth-Shaped Scaffold, Place Some Cells in Them and Let The Cells Grow**: It involves seeding of biodegradable scaffolding with cells and generation of these tissue will mould into the shape of that of scaffold. This step is based on the principle of tissue engineering and is very successful.

The well-documented 'ear on the back of a mouse' experiment carried out in 1997 by Vacanti and co-workers (Cao et al. 1997) is a vivid (if impractical) demonstration of the use of scaffolds[15]. The pioneering work of Shirley Glasstone-Hughes (1952) demonstrated and proved that the early-stage embryonic tooth primordia can be split in two and each half can generate a complete normal size tooth[16]. This established that early-stage tooth primordia have an inherent level of plasticity and regenerative capacity. This regenerative capacity was utilized in experiments by Young et al (2002) who, in collaboration with Vacanti, made scaffolds in the shape of different teeth and seeded these with cells dissociated from early-stage third molar tooth germs from pigs and rats[17].

The seeded scaffolds have been grown in the omentum of immune compromised rats, and histological evaluation revealed the formation of tiny (1–2 mm) tooth crowns 20–30 weeks after *in vivo* Implantation using porcine tooth buds[17]. The rat molar tooth germ, however, formed after 12 weeks of *in vivo* implantation[18]. The interpretation was based on the results that they establish the existence of stem cells in tooth primordia which are being able to regenerate teeth[18].

In fact, the concept behind this is that small numbers of dissociated dental epithelial and mesenchymal cells recombined in the scaffold and initiated tooth formation in a process suggested by the Glasstone- Hughes experiments[16]. This hypothesis is further supported by the fact that the teeth produced were tiny and thus probably formed from small groups of epithelial and mesenchymal cells reaggregating from the dissociated population. The drawback is that the mini-teeth that developed did not adopt the shape of the scaffold and bone was not formed in the process.

A Bio Tooth process must involve the formation of new bone into which the tooth can attach and develop its roots. These experiments have proven the remarkable ability of early dental cells to reorganize themselves and this itself may have potential uses in Bio Tooth production.

4. **Reproduce The Same Embryonic Development in the Oral Cavity**: this is a simple and precise process because complex organs are produced in the embryo and in vitro too.

The concept of third dentition is definitely an attractive concept. This is presented in terms of adding molecules to induce de novo tooth initiation in the oral cavity following tooth loss. Such molecules might be from embryonic tooth induction or successional tooth formation. The identification of mutations in *RUNX2* causing cleidocranial dysplasia, in which patients have a third set of teeth, has attracted attention as a possible route towards creating Bio Teeth[19]. The idea that *de novo*activation of genes such as *RUNX2* might be used to induce new tooth formation in the adult mouth does, however, pose obvious dangers as *RUNX2* plays a key

role in other cellular processes, including bone formation. In addition to this, another drawback with this type of approach is that the cells from which teeth develop are not present in the adult jaw, and thus there is 'nothing' for any inductive molecules to act upon.

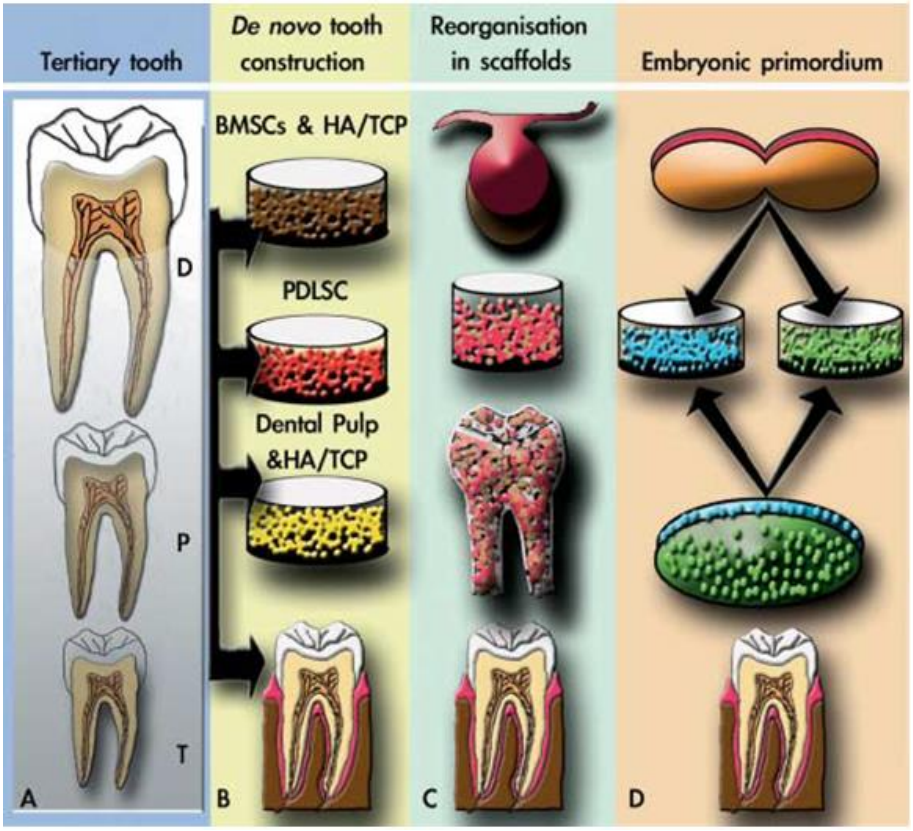
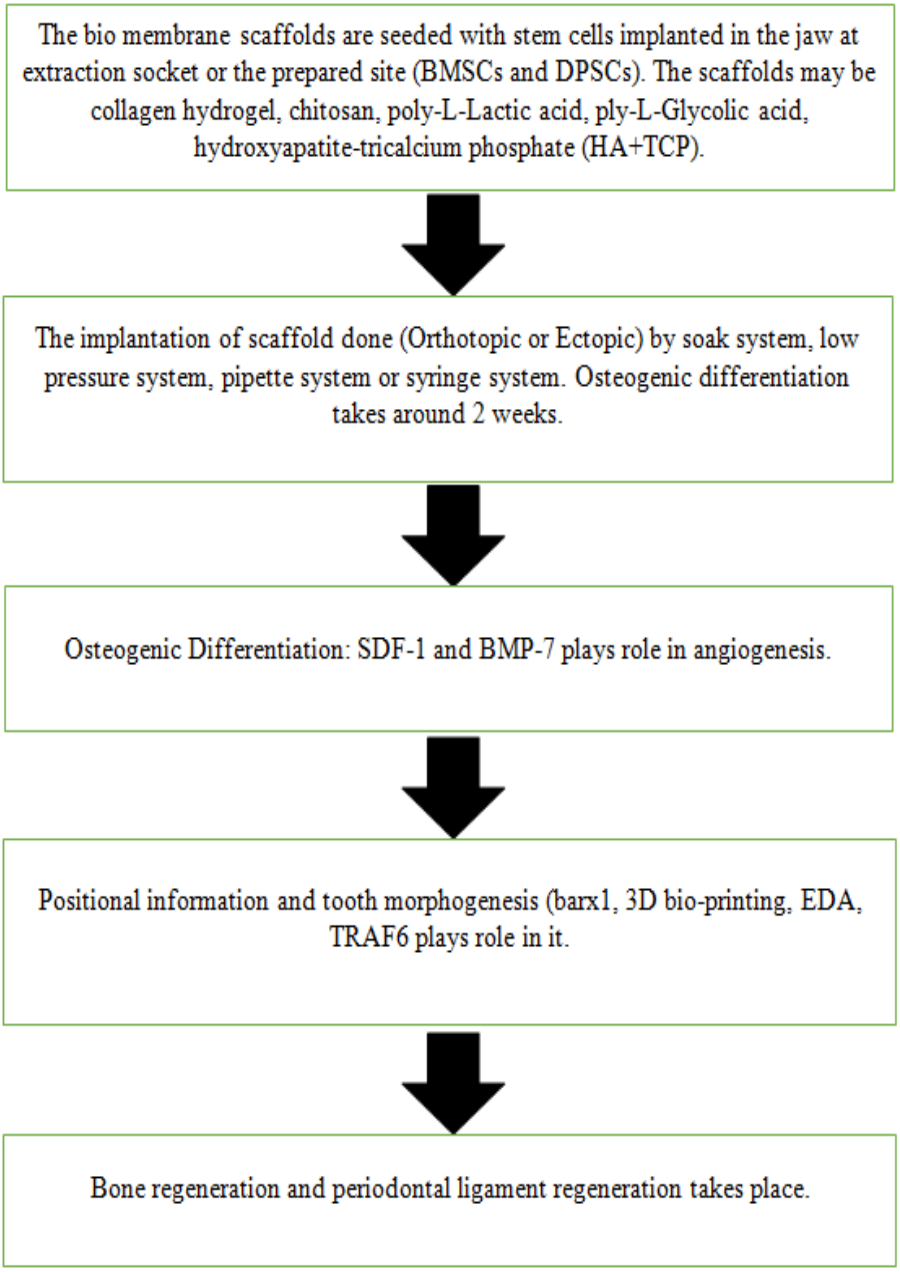



*Figure 4: representation of four different possible approaches to tissue engineering teeth. (A) Stimulation of third dentition(tertiary tooth) (Otto et al. 1997). (B) Construction of an adult tooth de novo (Robey, 2005). (C) Seeding of dissociated third molar tooth bud cells into tooth shaped scaffolds (Young et al. 2002; Duailibi et al. 2004). (D) Generation of a tooth primordium from cultured stem cells (Ohazama et al. 2004). T: tertiary; P: primary; D, deciduous*[1].

## Mechanism Involved In the Transformation of Xenodontics to Biodontics

Xenodontics is the type of dentistry of the restoration, repair and replacement of lost and damaged teeth using nonbiologic materials such as metals and plastics. Biodontics, whereas is the development of tissue-engineered tooth derived from stem cells. These are the biologically derived replacement of lost and missing teeth[20].

The process of Biodontics involve[2,9]:




## Reconstruction of Biotooth

## Recombination Experiments

In the developed countries, an estimated 7% of general population have lost one or more teeth at the age of 17 years. After the age of 50 years, an average of 12 teeth have been lost. World Health Organization (WHO) databanks showsthatdental caries remains widely spread in most countries worldwide (100% incidence in few populations); severe periodontal diseases can result in tooth loss are estimated to affect 5–20% of most adult populations, and the incidence of complete edentulism has been estimated between 7% and 69% internationally. Bio toothmay be reconstructed with the aid of using dental cells recombined with or without scaffolds, by pre/post-natal dental cells, or even

with nondental cells. Nakao et al. have validated that bioengineered incisor tooth germs may be reconstituted by the use of absolutely dissociated dental epithelialand mesenchymal cells in a three-dimensional collagen gel[22]. These bioengineered tooth germs can replicate the embryonic tooth organogenesis and develop into the wholeincisor in vitro or within the dental alveolus of adult mice.

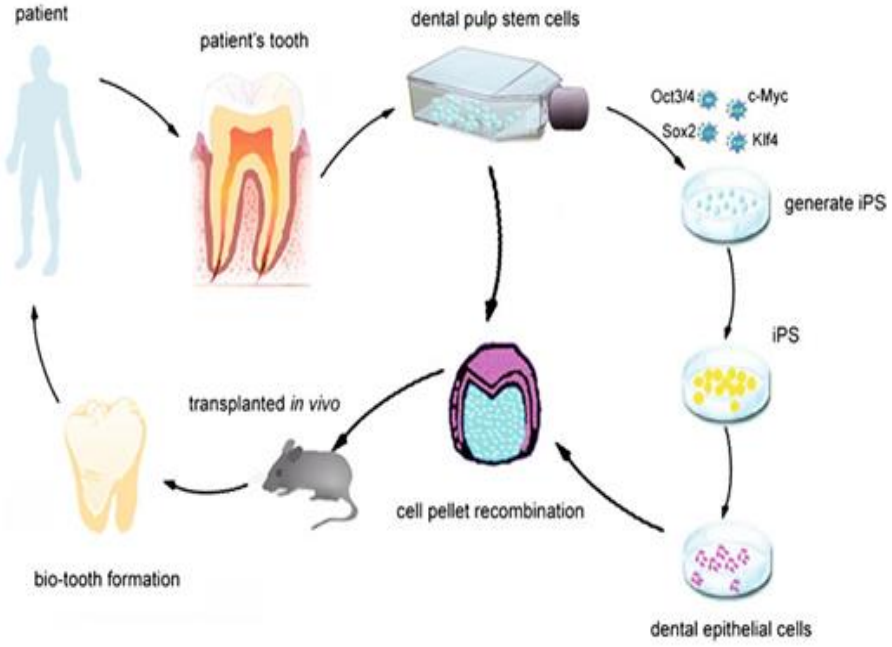



***Figure 5:*** *Schematic diagram illustrating the making of a bio-tooth using dental iPS cells. Autologous DPSCs are isolated from the patient's own dental pulps. Pluripotent iPS cells are created by driving four genes (c-Myc, Klf4,Oct3/4,and Sox2) intoDPSCs, which can be used to generate dental epithelial cells under suitable conditions. Then these iPS-derived dental epithelial cells are recombined with autologous DPSCs pellet and transplanted in vivo to make a bio-tooth. After the temporary incubation in vivo, bio-tooth can be transplanted into the patient's jaws to treat tooth loss*[21]

.

The tooth formation starts from the first interaction between oral epithelium and neural crest-derived mesenchymal cells in jaw primordia. The mesenchymal cells are differentiated in 6 weeks in humans and can respond to different ways to the epithelial signals. This stage is the one to reproduce in vitro from cultured cells. The dental epithelial cells form the ameloblasts of the tooth while the mesenchymal cells formthe different cell types, which includes odontoblasts, cementoblasts, pulp cells and periodontal ligament.

Challenges faced in this procedure are the identification of cell populations that can replace neural crest derived embryonic membrane and interact with oral epithelium to form mesenchymal cell types of the tooth.

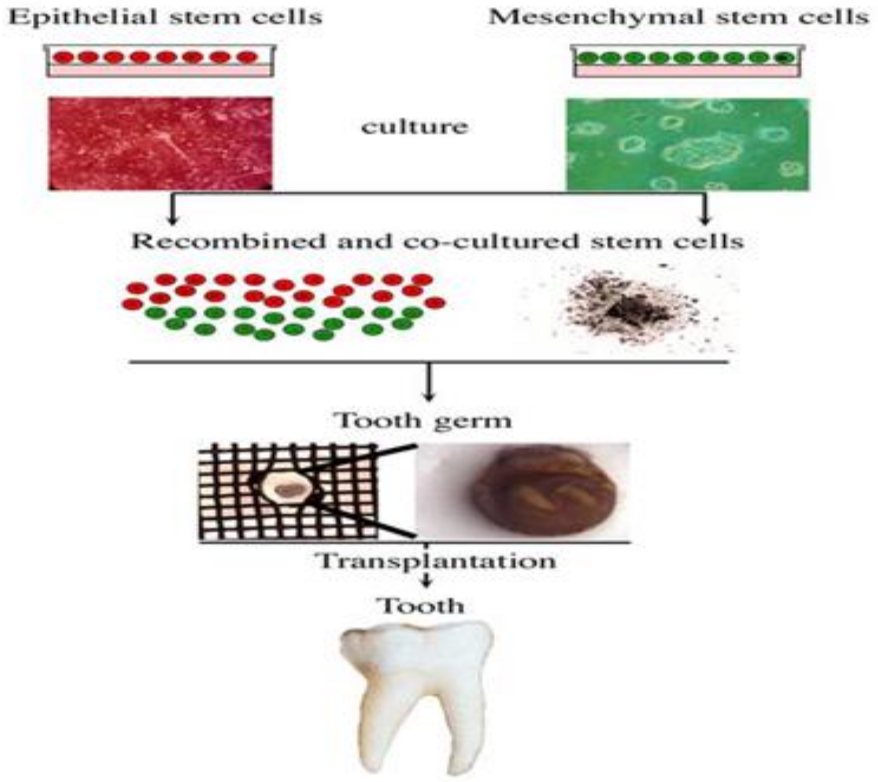



***Figure 6:*** *Use of stem cells for tooth formation in vitroand ex vivo. A tooth germ can be created in vitro after co-culture of isolated epithelial and mesenchymal stemcells. This germ could be implanted into the alveolar bone and finally develop into a fully functional tooth*[23].

This is the oldest method to aim at epithelial-mesenchymal interactions required for cell differentiation and tissue regeneration. There are three types of recombination methods between epithelial and mesenchymal components—tissue-to-tissue, cellto-tissue, and cell-to-cell recombinants. These can be incubated either in vitro or in vivo. At the beginning of odontogenesis, the ectomesenchyme seems to supply the initial inductive signals that are followed by the formation of dental placode.

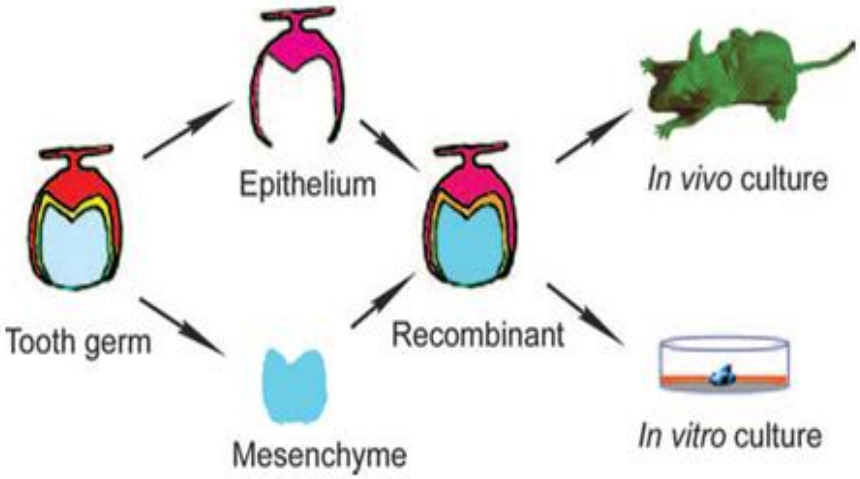


***Figure 7:*** *Developing tooth germ is isolated and separated into dental epithelium and mesenchyme. Then, two components are reassociated together to investigate the epithelial–mesenchymal interactions and odontogenesis either in vitro or in vivo*[21].

Subsequent cell proliferation, condensation, polarization, and differentiation of the epithelium and mesenchyme make a contribution to the tooth morphogenesis.

Several studies have demonstrated that tooth crown–like structures can be generated by recombination between embryonic oral epithelium and mesenchymal cells of dental or nondental origin, and even by neural crest cells in chick embryos.

These are an effective technique for bioengineering. The difficulty present is segregation of embryonic oral epithelium from underlying mesenchymal components. Therefore, this technique is of prime importance to clinical tooth regeneration.

## Scaffold - Based Tooth Engineering

This is the most popular approaching of making the bio tooth with the preformed scaffold. The scaffold should mimic the natural extracellular matrix environment. The ideal properties of the scaffold are providing chemical stability and features compatible to surrounding tissues such as adhesion performance, cell proliferation, controlled degradation and mechanical strength. Various scaffold materials used are natural occurring molecules of intermediate duration ( collagen and chitosan), to relatively short duration polymers such as polyglycolic acid (PGA), polylactic acid (PLA), polyglycolic acid-poly-L-lactic acid (PGA-PLLA), and polylactic polyglycolic acid (PLGA)[21].

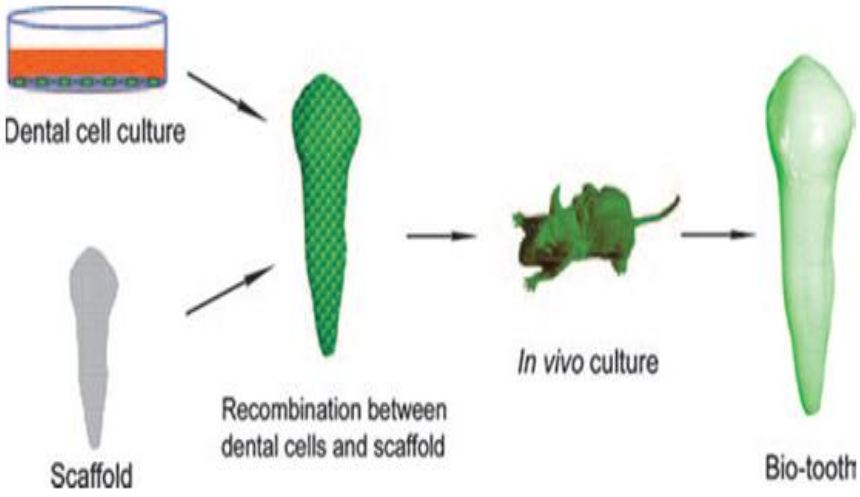



***Figure 8:*** *Schematic diagram for the scaffold-based tooth engineering. Tooth-like scaffold is recombined with dental cells and subsequently incubated in vivo to make a bio-tooth*[21].

Complex tooth-like structures have been generated by seeding dissociated tooth bud cells onto polyglycolic acid fibre mesh and other biodegradable scaffolds. Moreover, dental pulp stem cells (DPSCs)in conjunction with hydroxyapatite/tricalcium phosphate (HA/TCP) powder in vivo can form a dentin-like structure lining the surfaces of HA/TCP particles. When stem cells from apical papilla and periodontal

ligamentare recombined with HA/TCP, root and periodontal ligament– like complex can be generated in vivo.

There are many complications to the scaffold-based tooth engineering such as existence of scaffold has a negative impact on the epithelial-mesenchymal interactions and odontogenic environment. The artificial scaffold can become a hurdle for the natural cell-cell, cell-matrix interactions making it uncontrollable to sustain the shape and size of bio tooth. The acidic components present in the PGA, PLGA and PLA have an adverse effect on the dental tissues. The nutrition delivery and excretion of waste products is limited in scaffolds.

Limited calcification of ECM can only be seen in the ceramic scaffold. Therefore, the relationship between dental stem cells and scaffold materials should be further evaluated before their clinical use can be considered.

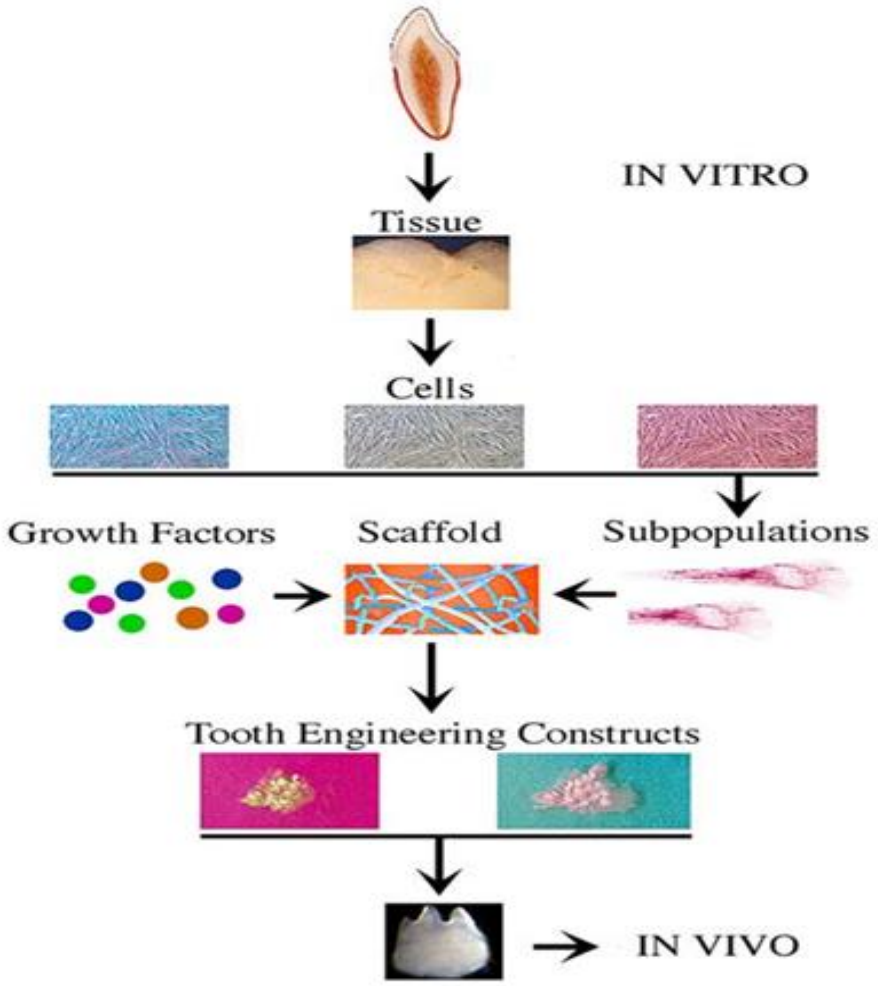



***Figure 9:*** *Construction of a bioengineered tooth. The association of tooth-derived stem cells with defined scaffolds in the presence of growth factors allows the creation of tooth specific constructs such as crown and root of missing parts of an injured tooth. These biological constructs could be used in dental clinics as substitutes for metal implants, crowns and restorative dental materials*[23].

## Cell Pellet Engineering

This is also called scaffold free method, aims at simplifying the complicated operating procedures of scaffold-based engineering and recombination experiments. The cells used in this are either dental mesenchymal cells alone or mixed tooth germ cells (mixture of epithelial and mesenchymal cells).

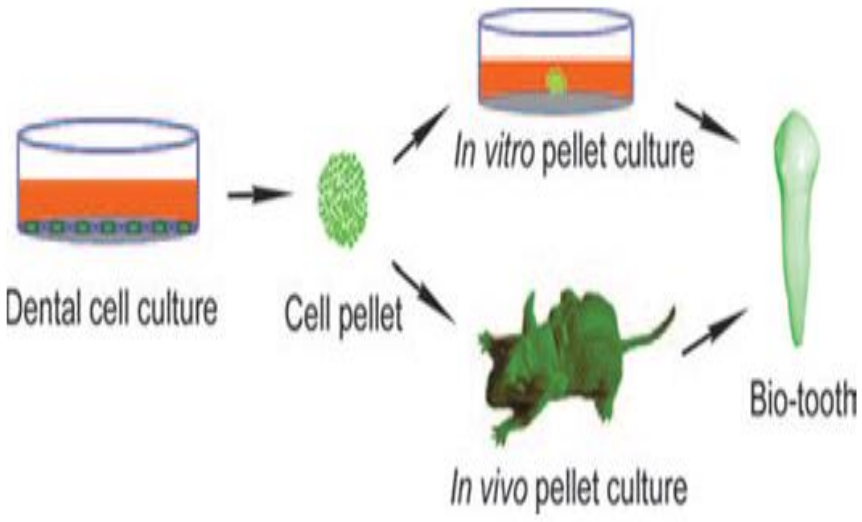



***Figure 10:*** *Schematic diagram for the cell pellet engineering. Dental cells (i.e., dental mesenchymal cells or mixed tooth germ cells) are precipitated for collecting the cell pellet, which is subsequently cultured in vitro or transplanted in vivo to produce the three-dimensional dental structures*[21].

The benefits of this method are better cell-cell and cell-matrix interactions, and sufficient cell movements and selective cell adhesion inside pellets. Thus, it is easy for each cell to find its position, perform self-reorganization and differentiation at a normal pace. This process is easy to operate as there are no additional materials used and no need to separate epithelial cells from mesenchymal cells.

The native extracellular matrix (ECM) disappears in this cell pellet engineering during primary cell isolation and trypsinization. The re-aggregated dental cells in three-dimensional pellets can secrete new ECM that act as a natural scaffold and generate bioactive factors necessary for bio tooth formation. With the help of this technique, dental papilla mesenchymal cell pellets (DPMC) with conditioned medium and dental epithelial/mesenchymal cell re-aggregations in vivo can create regular dental-pulp complex and tooth like structures. This process is more meaningful because our tooth is derived from different cell pellets.

## Chimeric Tooth Engineering

In clinical science, a chimera is an individual, organ, or part of an organhaving more than one genetically distinct population of cellsthat originate from more than one zygote/individual. This is widely used in the organ transplantation such as kidney, heart, liver and skin replacement. Nakao et al. have reported that the chimeric bioengineered tooth germ can be generated by embryonic epithelial and mesenchymal cells, respectively, isolated from normal and GFP-

transgenic mice. Many previous research has suggested that dental papilla cells from multiple incisor germs at the similar developmental stages can result in the formation of one dentin-pulp complex in the absence of dental epithelial components. This procedure has wide implications for tooth reconstruction, creating a chimeric tooth in a short period of time using dental papilla cells from multiple teeth or individuals.

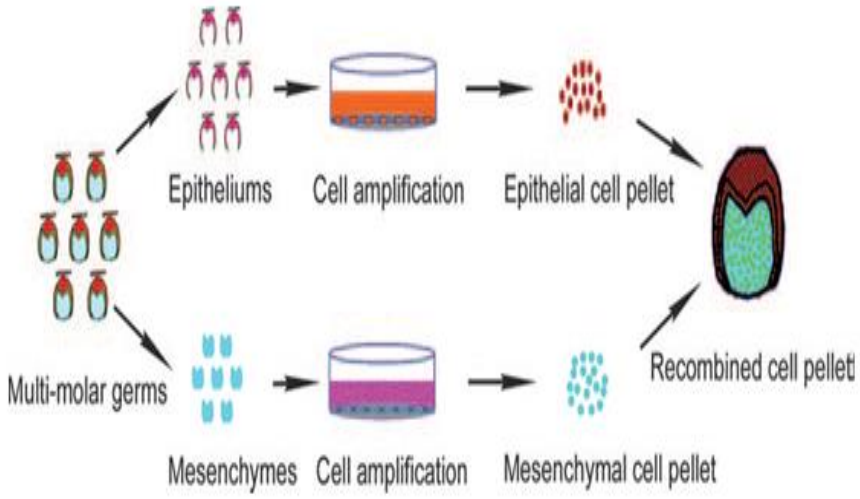



***Figure 11:*** *Schematic diagram for the chimeric tooth engineering. Dental epithelial and mesenchymal cells are obtained, respectively, from many tooth germs of different individuals at the same developmental stages to guarantee sufficient cell amplification at a relatively short time. Then, epithelial and mesenchymal cell pellets are collected, respectively, and reconstituted together to make a chimeric bio-tooth*[21].

DPSCs and BMSSCs from incisors and bone marrow are recombined with apical bud cells (ABCs) from a number of postnatal germs. After 14 days of incubation, DPSCs/ABCs chimera form tooth crown-like structures while BMSSCs/ABCs chimera bring about two dentin-pulp complex without enamel formation.

## Gene - Manipulated Tooth Regeneration

Gene based therapies modify the phenotypes of recipient cells by delivering specific gene into the target cells., stimulating recipient cells to differentiate into desired lineages. Development of the tooth is the process guided by many genes, directing tooth buds to form specific teeth. It works on two strategies. One is in vivo gene- manipulated odontogenesis, which means, endogenous dental cells in situ may be activated or repressed by gene delivery technique to make a tooth. This seems dangerous and impractical leading to certain mutations in genes. The other strategy is, in vitro gene-manipulated odontogenesis, which means, gene transfer technique may be used to reconstruct a tooth.

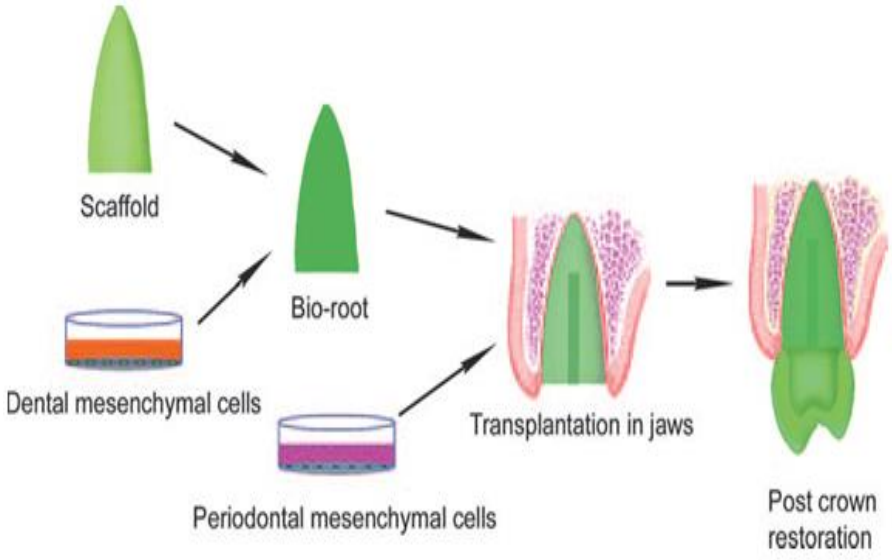



***Figure 12:*** *Schematic diagram showing the [hybridized] tooth root engineering. Dental mesenchymal cells are reassociated with the biodegradable scaffold to make a biological root (bio-root). The bio-root is subsequently recombined with periodontal mesenchymal cells and transplanted into the jaws. Finally, post crown restoration is performed to recover the original tooth function[21].*

Recent research has proved that gene transfer of growth/differentiation factor 11 (Gdf11)52 or bone morphogenetic protein-2 (BMP-2)53 can induce the differentiation of DPSCs into odontoblasts in vitro and stimulate the reparative dentin formation in the dog model. Thus, the inflamed pulp under deep caries or trauma, possibly due to the limited supply of pulp stem/progenitor cells, might be treated with the transplantation of these Gdf11-enhanced stem cells.It is beneficial as it provides a sustained delivery of growth factors at the physiologic levels. However, the system should be carefully evaluated to get rid of gene pollution.

## Engineering the Root and Periodontal Complex

There are a series of events happening in the formation of root-periodontal complex, namely, instruction of Hertwig's Epithelial Root Sheath (HERS), formation of periodontal ligament, tooth eruption, dentinogenesis, cementogenesis and osteogenesis involving rootsheath cells, papilla cells, dental follicle cells, odontoblasts, cementoblasts, osteoblasts and periodontal ligament cells. The aim of the reconstruction is to find a replacement of the loss by dental implants. Dentists can but biological crowns on the bio root to restore the damage of tooth loss. Young et al. recombined tooth bud cells and bone marrow progenitor cells with biological scaffolds to generate bio-tooth and bio-bone, respectively. These resulting tissues are subsequently sutured together to produce the hybrid tooth–bone tissues containing periodontal ligament and root structures. Hu et al. have reported that the recombinants between dental epithelial and mesenchymalcells from mouse embryos can generate roots, periodontal ligament, and surrounding bone in the subcutaneous area behind the mouse ears. The most feasible approach is hybridized tissue engineering by Sonoyama et al. This research team integrates several methods together to recover tooth functionand

appearance, including stem cell–based tooth regeneration, biomaterials, and crown restoration techniques. When stem cells from apical papillae and periodontal tissues are recombined with HA/TCP ceramic particles, bio root– periodontal complexes are formed in vivo that can support a porcelain crown and bring about the normal masticatory and aesthetic functions.

The strategy for the periodontal ligament regeneration is cell-sheet engineering. Temperature-responsive culture dishes made of polymer poly (N-isopropylacrylamide) are used to create the cell sheets. Under normal culture conditions at $37^0$C, the dish surfaces are relatively hydrophobic, in which cellsattach, spread, and proliferate similarly to those on typical tissue culture dishes. However, when surrounding temperature is below the polymer's lower critical solution temperature of $32^0$C, the polymer surface becomes hydrophilic and swells, forming a hydration layer between dish surface and cultured cells. Then, cultured cells are spontaneously detached from the dishes without the need of enzymatic treatments.

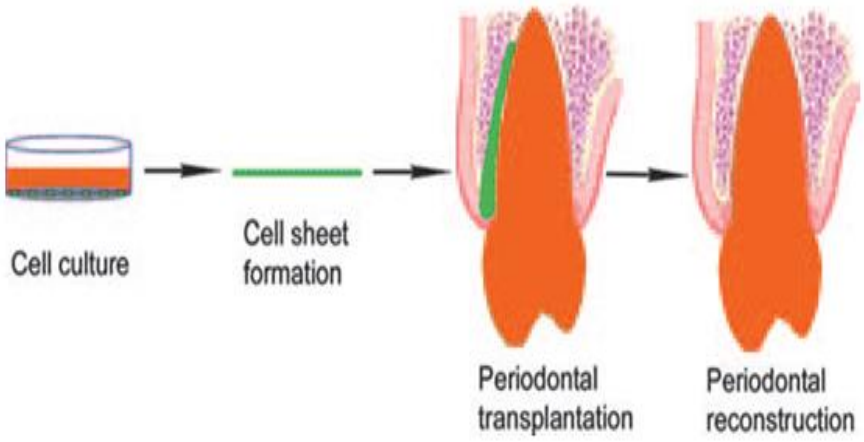



***Figure 13:*** *Schematic diagram for the cell sheet engineering. Cell sheet is isolated from the temperature-responsive culture dish and transplanted in vivo to realize the periodontal reconstruction*[21].

Supporting periodontal apparatus such as cementum, periodontal ligament and lamina dura (inner layer of the alveolar bone proper) are reconstructed by this technique. This method cannot be applied in areas of severe bone destruction and large periodontal defect due to limited cell layer in the sheet system.

## The Right Shape & Size of Biotooth

It is crucial that bio tooth acquires right shape and size according to the natural tooth morphology. Tooth shape is primarily determined at the early odontogenesis. The formation of the shape of the bio tooth is either the result of the prepatterned cranial neural crest derived mesenchymal cells (CNCCs) or the consequence of the response generated by the oral epithelium due to CNCCs. The proportions of dental mesenchymal and epithelial cells can affect the regular shape of bioengineered teeth, and this may provide a preliminary study toward the determination of bio-tooth shape[10,12].

Several secreted signalling molecules, such as BMPs, FGFs, Wnts and Shh, are expressed in the epithelium and function as morphogens that control the

generation of diverse tooth shapes. For example, BMP4 expression is linked with the incisors' shape, while FGF8 is linked with the shape of molars. BMP4 activates expressionof *Msx1* and *Msx2* in the mesenchyme of future incisors. Similarly, *Islet1* is expressed only in the epithelium of the incisors and its expression is regulated by BMP4. By contrast, FGF8 activates *Dlx1*, *Dlx2* and *Barx1* expression in the mesenchyme of future molars[12].

Furthermore, the shape of maxillary and mandibular teeth differs and is controlled by genes such as *Dlx*, *Barx1* and *Pitx1*. For example, *Pitx1* deletion affects only the mandibular molars, which are smaller and have fewer cusps[13]. Alteration of the odontogenic signalling cascade might also lead to modification of tooth size. For example, smaller teeth were reported in mice after deletion of Wnt signalling[12].

## Root and Eruption of the Biotooth

The successful reconstruction of the bio engineered tooth requires formation of the correct shape and length of the root. For example, short roots have a difficulty in retention of bio tooth. It is also necessary that structures related to the root like periodontal ligament should remain functional for longer periods, avoiding the risks of ankylosis[10]. The root and tooth eruption are time consuming processes in bio tooth. The immunological rejections in the patients should be avoided by taking the similar tissues/ tissues from the same individual[11].

## Present Challenges Associated with Biotooth

### 1. Controlling The Shape and Size of Biotooth

Scaffolds are mainly used for this purpose. There is less evidence that scaffolds can control shape and size of the bio tooth. In the report of Younget al., PGA-PLLA/PLGA scaffolds in the shape of humanincisors and molars are seeded with $2.0*10^6$ postnatal toothbud cells, and then implanted into the omentum of athymicrats[17]. Histological analyses have revealed that only a smalltooth crown, approximately 2mm by 2 mm, is formed in thetooth-shaped scaffolds with the original dimensions of1.0 cm by 0.5 cm by 0.5 cm.Sequential seeding of dissociated epithelial and mesenchymal cells on the surface of thecollagen sponges (approximately 11mm in diameter and2mm in thickness) results in the formation of bio-teeth witha size of only 1 mm. therefore, scaffolds are impractical approach to sustain the shape and size of the bio tooth.

### 2. Postnatal Epithelial Cells Necessary for Making The Biotooth

Another challenge which arises is the insufficient donor tissues present, mainly from the third molar germs and the low ex vivo expansive potential of epithelial cells. Alternative sources meeting this criteria are to be used . Hu et al. have proved that bone marrow–derived cells can be driven into ameloblast lineages with polarized appearances. Another alternate is chimeric tooth engineering in which the shape of the bio tooth is determined by the mesenchymal cells and epithelial cells will disappear.

### 3. Graft Rejection During Bio-Teeth Transplantation

The ability to differentiate self-antigens from non-self-antigens is known as major histocompatibility complex (MHC). In humans, it is also known as human leukocyte antigen (HLA). Graft rejection takes place due to immunological responses by the non-self-antigens. If the donor tissue is taken from a different species or unrelated host, HLA are most likely to be different. The host's immune response exacerbates as it takes the graft as foreign and expresses robust immune responses against it. T lymphocytes, mainly responsible for cell-mediated immunity are released and attack the transplanted tissue and destroy it in a short period.

Tissues from the patient's own cells do not cause risk, but the ex vivo incubation of bio-teeth may cause a potential infection and rejection. Therefore, this is another problem faced by bio tooth.

### 4. Growth of Biotooth in Jaws

There are three ways to generate bio tooth- in vitro, in vivo and ex vivo or heterotrophic sites. Several heterotrophic sites are omentum, renal capsule, anterior chamber of the eye, embryonic chicken etc. although, all of them are quite impractical when compared to patient's own tissues.

Ohazama et al.have proved that embryonic tooth primordia can maintain the normal developmental process and bring about the tooth formation when transplanted into the diastema region of adult mouse mouth[24]. When dissociated canine molar tooth bud cells are recombined with a biodegradable polymer and transplanted into the same alveolar sockets where the tooth buds have been extracted, tubular dentin and new bone can be regenerated in the jaws, while no amelogenesis and cementogenesis can be detected in these recovered recombinants. Nakao et al. further demonstrate that a bioengineered primordial organgenerated from recombined dental epithelial and mesenchymal cells can replicate the embryonic tooth organogenesis in the dental alveolus of adult mice, which opens up the new exciting prospect of bio-teeth in future clinical applications.

Many problems can occur before transplantation of bio tooth in patient's mouth such as graft rejection, insufficient blood supply in jaws, contamination by saliva, and guiding signals from the surrounding tissues during the tooth growth and eruption. Therefore, it is imperative to have the right signal at the right time.

### 5. Eruption of The Biotooth from Jaws

The most challenging problem is the proper eruption and guidance of bio tooth in the right place in the oral cavity. The mimicking tooth can only be formed if the dental follicle or dental sac is intact. Larson et al. have shown that teeth without dental follicles cannot erupt, but teeth that are recombined with dental follicles can erupt. Therefore, for the success of bio tooth reconstruction, dental follicle should be the candidate marker in this process[21].

## Future Perspectives

DPSCs plays prime role in tissue regenerations, though its immediate application is of main concern. Autologous DPSCs collected from the dental pulp of permanent teeth can be used for different therapeutic purposes. Future goals may consider and focus upon mechanism of differentiation of these dental stem cells, organisation of bio-scaffolds and exploring the suitable environment for odontoblastic differentiation. Several issues involving in the making of a stem cell-mediated bio-tooth must be solved, including identification and 'stemness' maintenance of stem cells, dental morphogenesis, tooth type determination, odontogenic signal cascades, odontogenic epithelium availability, controllable bio-tooth growth and eruption, pulp revascularization and neural regeneration, and host-graft immune rejection in the jaws.Furthermore, the use of culture-expanded stem cell population needs to take into account the possibility of genetic and epigenetic instability.In addition to this, tumorigenesis, use of retroviruses and xenogeneic materials need to be addressed by further qualitative research in this field[25].

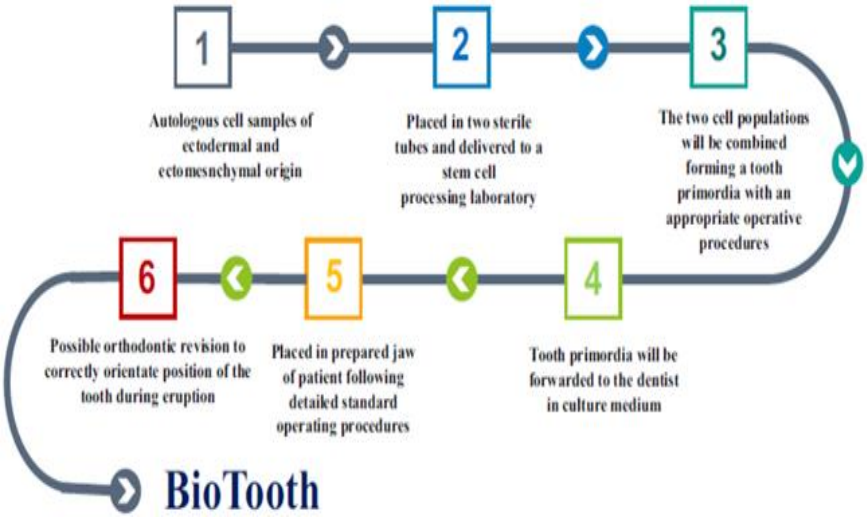



***Figure 14:*** *Linear flow chart representing the concepts and future perspective strategies creating a biological tooth*[26].

## Summary & Conclusion

Dental regeneration is one of the most exciting areas of development in dentistry. Dental stem cells have clinical applications both in medicine and dentistry. Tooth regeneration provides an attractive alternative to the increase in failing dental implants. The concept relies on the in vitro recreation of the genetic odontogenic program using stem cells. Currently, scientific advances in developmental and molecular biology, experimental embryology, molecular genetics, stem cells biology, bionics and biotechnology have provided a number of opportunities to realise the tooth reconstruction. The concept of bio engineered tooth is relatively recent and currently under research. Various problems should be overcome to translate this novel therapy from laboratory to clinics.

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