REVIEW ARTICLE

Regenerative dentistry: Current status and future scope

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Abstract

Regenerative dentistry is a rapidly evolving treatment approach in dentistry intended to bring back the original structure and function of defective tissues. The basic strategy is to utilise the inherent regenerative potential of progenitor cells within dental tissues through the prudent use of biomaterials-based scaffolds. Regenerative dentistry has evolved through decades of systematic observations, case studies and biomaterial development. The core concepts of regenerative medicine and tissue engineering were incorporated to improve the predictability of new tissue formation. Regenerative approaches have led to the development of novel functional biomaterials, biomolecules and engineered scaffold systems with promising potential towards regeneration. In this scenario, a thorough knowledge of the dental progenitor cell populations and their responses to various biomaterial systems is invaluable. In addition, the isolation and characterisation of tooth-derived cells and the development of *in vitro* cell culture systems can provide an immense advantage in the understanding of host tissue responses to biomaterial-based scaffolds. This review is an attempt to describe the established methods and evolving concepts of dental tissue regeneration. In the initial section, various dental tissues are introduced, along with the challenges encountered in the management of tissue defects. The conceptualisation of dental tissue regeneration and the conventional regenerative approaches of dental pulp and periodontium are explained in the following section. Further, the methods of harnessing the regenerative potential of the tooth-derived cells are provided. The types of tooth-derived cells, procedures for isolation and culture of the cells and the importance of their characterization and differentiation, are described in the subsequent sections. Thereafter, endogenous regenerative approaches using conventional and bioactive biomaterials, biofunctionalized matrices and novel biomaterial systems are discussed. The review concludes with a note on the future directions towards novel biomaterial designs and cell based methods for dental regeneration.

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Introduction

The developments in Science, Technology and Biology that occurred in the past decades drastically changed the face of health care. Dentistry is an allied area to Medicine which has assimilated this change and achieved tangible outcomes in recent times. A paradigm shift could be observed in the management of tissue damage with the advent of Tissue Engineering techniques that culminated in the new approach of 'Regenerative Dentistry'. This article reviews the background and development of this area and presents the current status.

The sections below cover the essentials of dental tissue architecture, challenges that prompted the adoption of regenerative approaches, conventional management of periodontal and pulpal damages, the role of tooth-derived stem cells, the role of biomaterial scaffolds and new approaches towards tissue regeneration. This review explores the conventional and modern approaches in Regenerative Dentistry and looks into the future scopes. Stress is given to the regenerative approach pertaining to periodontal and pulpal tissue defects, and the role of cells and biomaterials-based scaffolds.

Dental tissues

Dentistry deals with the tissues related to the tooth and supporting structures of the oral cavity to enable the primary function of mastication. The dental tissue structure consists of both soft tissues and hard tissues organised in a highly anisotropic architecture. This specific structure makes it possible to take the heavy and transient loads of mastication and helps to withstand the mechanical, chemical and biological stimuli in the oral environment. The heterogeneous and specific cell populations distributed within the dental tissues take care of the formation and maintenance of tooth structure [1].

The framework of the tooth is formed by the hard tissue 'dentin', which is resilient and strong in nature. It is composed of dense collagen fibres arranged across the thickness, aligned in hollow tubular architecture. This collagen structure upon which calcium hydroxyapatite crystals are incorporated to 70% w/w, provides hardness and resilience to the tooth. Within the dentin frame inhabits the 'dental pulp', the vascularized and innervated vital tissue that helps in the deposition and maintenance of dentin. The blood vessels and nerves to the pulp enters from the host alveolar bone through the narrow apical foramen at the root end of the tooth. The association of dental pulp and dentin covering is quite complex, with the dentin protecting the dental pulp within, and the dental pulp depositing new dentin continually [1, 2].

The upper part of the tooth protruding to the oral cavity is called the 'crown', where the underlying dentin framework is covered by enamel, the hardest material in the human body. The hardness of dental enamel arises out of the unique dense arrangement of fluorapatite mineral (up to 96% w/w), with minimal organic content. Unlike dentin, enamel does not contain collagen; instead, the mineral part is held together by two unique classes of proteins - amelogenins and enamelins [1].

The root part of the tooth anchors to the alveolar bone (maxilla and mandible) with the aid of the 'periodontal ligament'. The root dentin of the tooth is covered with a hard lining called 'cementum', to which periodontal ligament fibres are attached so that the tooth is firmly held within its socket in the alveolar bone. This, along with the dense collagen fibres called Sharpey's fibres, helps in transmitting and dissipating the masticatory forces of the range 400 N [3]. The part referred to as the 'periodontium' contains four distinct compartments – cementum, alveolar bone, periodontal ligament and gingiva.

Challenges to dental tissues and their management

The teeth and associated dental tissues are very complex in architecture compared to any other part of the body, and it requires specific and in-depth understanding and specialised techniques to maintain dental health. Moreover, due to their position and function, dental tissues are highly prone to defects and damage, primarily by microbial insults or traumatic events. These aspects made dentistry come up as an allied field of medicine.

Diseases of dental tissues are dealt with, two approaches – one is 'repair' of damaged tissues and the other is 'regeneration' of the local tissue structure. Repair refers to the healing of defects that progresses through the growth of fibrotic scar tissue and ectopic calcifications. Though the disease condition is managed, this will not completely reinstate the intricate microstructure or help to regain the functions fully. Regeneration aims at the

complete restoration of function and structure of the lost tissues, thereby facilitating normal biological activities [4]. Achieving complete regeneration is a tougher task compared to repair (and rather hypothetical in certain cases), because it requires deeper knowledge and specific techniques to engineer the tissue formation.

Regenerative dentistry

The attempts at dental tissue regeneration started in the 1980s and gained acceptance through the 1990s. The techniques are being constantly modified with the advancements in novel biomaterials and treatment strategies since the dawn of the 21st century. During these years, a paradigm shift from the conventional reparative treatment approaches to strategic regenerative approaches evolved in dentistry, backed by a renewed understanding of infection control and the role of host progenitor cells in attaining regeneration [5]. This knowledge brought positive outcomes in the management of both pulp infections and periodontal defects. Later, with the advent of Tissue Engineering techniques and regenerative medicine, it was possible to plan engineered regeneration of specific tissues and structures, which led to the emergence of Regenerative Dentistry.

Regenerative dentistry attempts to understand the biological processes involved in tooth development, healing, repair and regeneration and utilises the knowledge to devise novel clinical treatment approaches towards regaining structural and functional aspects of damaged dental tissues [1,6]. Thus, by applying the principles of regenerative dentistry, predictable regeneration of dental tissues can be achieved through the activation of inherent resident stem cells and the modulation of their responses to immediate environmental cues such as ECM in the presence of biomaterial scaffolds, mediators and growth factors.

Conceptualisation of tissue regeneration

The concept of dental tissue regeneration evolved through a better understanding of the embryonic development of the tooth, postnatal tooth organogenesis, and the cellular and molecular interactions between the cells and the ECM [6]. The control of inflammation, removal of diseased tissues and allowing selective repopulation of appropriate cells in the disease area were understood as key factors for periodontal regeneration [7,8]. The concept of periodontal regeneration was adopted in the regeneration approaches of dental pulp and periodontium by the prudent modulation of the host environment towards regeneration using suitable biomaterials [4,5]. Regeneration of dental tissues can be attained by utilising the inherent regenerative potential of the resident stem cells and by carefully modulating the interactions between the host stem cells, growth factors, mediators, and their immediate extra cellular matrix (ECM). The literature shows that the techniques were mainly applied to the defects of periodontium and pulp-dentin complex.

Conventional regenerative approaches

Conventionally, the regenerative approaches were considered as an alteration of reparative responses that may occur when adequate biological conditions co-exist in the host tissues [4-8]. The increased deposition of collagen and formation of reactionary dentin by the odontoblasts in case of mild inflammation of dental pulp in response to enamel caries extending to dentin is an inherent regenerative mechanism, whereas the formation of reparative and sclerotic dentin by odontoblast-like cells becomes a reparative response as inflammation progresses [9]. Conventionally, both mechanisms would be considered regenerative, but only the former response is truly regenerative because the original structure and function of dentin will be preserved [10]. Similarly, in periodontal regeneration, only a true ligamental attachment between the cementum and alveolar bone can be considered a regenerative response, whereas epithelial healing alone will be an undesired re-

parative mechanism [7,8,9].

The vital dental therapies such as indirect pulp capping, direct pulp capping, pulpotomy, apexogenesis, pulpectomy and apexification, earlier revascularisation attempts etc., can be considered conventional methods of attempted regeneration of dental pulp [9,10]. The use of non-resorbable barrier membranes to permit periodontal regeneration is also an earlier regenerative approach that necessitated an additional surgical procedure to remove the barrier used [8]. These conventional approaches have contributed to further developments in regenerative dentistry by establishing the basic principles and reiterating the possibility of the inherent regeneration ability of dental tissues.

Regeneration of the periodontium

The main disease of periodontium- infection episodes, known as 'periodontitis', results in loss of attachment of the teeth to the supporting alveolar bone due to the damage to periodontal ligament fibres composed mainly of collagen. If the infected region is cleared of the infection and associated debris through debridement and microbial control, there is a chance for natural regeneration to commence [7]. In the process, the reparative response can override, and the gingival epithelium tends to overgrow into the defect area. This prevents the reattachment of periodontal fibres, leading to mobility and the ultimate loss of the tooth. The nature of new attachment formation depends on the type of cells repopulating the post-surgical periodontal defect area (Melcher's Hypothesis) [8].

This knowledge led to the use of barriers in the form of resorbable membranes over the defect sites to prevent epithelial migration and to promote selective repopulation of the periodontal ligament cells. A biological environment will be created to form the ligamental attachment between the tooth root and alveolar bone. The combinatorial treatment approach of using alveolar bone grafts, bone graft substitutes and barrier grafts further promotes alveolar bone regeneration. This 'Guided Tissue Regeneration (GTR)' is a typical example of replacing a reparative response with a regenerative treatment approach to regenerate the periodontium structurally and functionally [7, 8].

Regeneration of the Pulp and Dentin

The dental pulp is enclosed within the hardcover of dentin and, as long as it remains vital, manages the nutritive, sensory, reparative and regenerative functions [9]. The dental pulp consists of specialised cells called the odontoblasts that lay down dentin in tubule stacks across. The odontoblast cell bodies line the inner boundary of dentinal tubules and form the outermost layer of dental pulp [10].

The dentin part, in case of any incidental damage, gets repaired naturally as long as the odontoblasts from the dental pulp are available [11]. Otherwise, a repair process will be initiated by the stem cells within the dental pulp. The nature of reparative dentin will be irregular and non-tubular and cannot be considered a truly regenerative process, even though it stabilises the structure and function of the teeth [12]. The regeneration of dental pulp is assessed by the quality and quantity of the dentin it secretes. Even if a remnant of healthy dental pulp tissue remains, dentin formation and continual dentin deposition can be induced using appropriate biomaterials, biologics, or a combination. This method of vital pulp therapy is called 'dental pulp capping' [13]. Dealing with an irreversibly inflamed or infected tooth before the completion of root formation is clinically challenging. The dental pulp needs to be removed to prevent the spread of infection to the periradicular tissues. The root canal space could be supported by making a blood clot formed by bleeding induced into the pulp chamber through the apex, which is known as revascularisation or revitalisation [14]. The tissue thus formed within the dental pulp space could be pulp-like if remnant apical papilla cells or dental pulp cells are available. Otherwise, the tissue formed will be periodontal ligament-like, with cementoid/osteoid tissues within the dental pulp [4,6].

Harnessing the potential of the tooth-derived stem cells

Stem cells are the undifferentiated cells of multicellular organisms capable of undergoing division resulting in cells of the same type, as well as differentiating into cells of various lineages [15]. The human tooth and the associated soft tissue architecture also harbour healthy populations of stem cells within. These cells termed the **'tooth-derived stem cells'**, have their origin mainly from the cranial neural crest cells formed during their embryonic development, and they retain this neural crest cell lineage throughout adulthood [16]. This fact accentuates the importance of tooth-derived cells, as almost all major human organs contain cells and tissues derived from the neural crest cells, often called as the fourth germ layer [17]. The embryonic developmental processes that are initiated *in utero* are continued further postnatally because of the continued development and successive eruption of teeth till adulthood [6]. The dental stem cells are reported to differentiate into osteo/odontogenic, chondrogenic, neurogenic and myogenic lineages [17-19].

The native stem cells in dental tissues

Stem cells and progenitor cells are found in all the soft tissues associated with the tooth. The dental pulp and periodontal ligament of permanent and deciduous dentition, gingiva, apical papilla and dental follicle during the formative stages of teeth harbour populations of stem cells that can be termed tooth-derived stem cells [16 - 19]. This stem cell population is responsible for the regeneration of dental tissues in case of microbial and traumatic insults. These tooth-derived stem cells have been isolated and utilised for various purposes right from the beginning of this century [18]. Figure 1 indicates graphically the various source tissues from where the different populations of tooth-derived stem cells are obtainable.

The cell responses can be effectively modulated by the prudent use of biomaterials and treatment strategies towards selective repopulation of cells in cases of guided tissue/bone regeneration (GTR/GBR) procedures. Biomaterial based modulation of cellular responses can also induce new dentin formation and regeneration of dental pulp. In addition to the stem cell population, the dental tissues also harbour a heterogeneous population of fibroblast cells, immune cells, neuronal cells etc., which aid the routine homeostasis of the dental tissues [20]. The successful isolation and culture of tooth-derived stem cells from

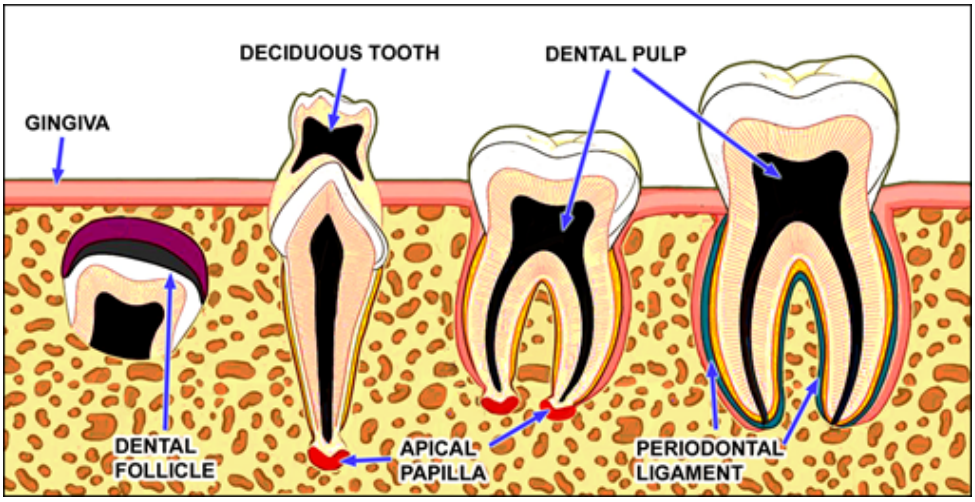

Fig 1: The various source tissues, out of which the tooth-derived stem cells are obtainable

all possible dental tissues are currently well-reported and practised worldwide.

The types of tooth-derived stem cells

The tooth-derived stem cells can be isolated from the dental pulp, periodontal ligament, dental apical papilla, dental follicle, and remnant tissues of exfoliated deciduous teeth, and the gingiva. The principal advantage of tooth-derived cells is the ease with which they can be procured, compared to other tissues in the body. The tissues necessary for the isolation of tooth-derived cells are in easily accessible locations within the oral cavity and can be obtained through routine dental procedures such as dental extraction, gingivectomies, surgical removal of impacted teeth, exfoliated deciduous teeth, biopsies etc. [18, 19].

The periodontal ligament contains a heterogeneous cell population capable of differentiation to cementoblasts (cementum forming cells), osteoblasts (bone-forming cells) and fibroblasts that lays down and remodels collagen [21]. The dental pulp harbours the dental pulp stem cells that maintain continued dentin deposition and maturation [17]. Gingival stem cells predominantly consist of fibroblast like stem cells, with a minor fraction of epithelial cells. The stem cells from apical papilla (SCAP) maintain continued root dentin deposition following tooth eruption till the root formation is complete, after which the apical papilla regress. The dental follicle stem cells (DFCSs) are present in the dental follicle during the formation of teeth, especially third molars. Due to the differential eruption patterns of teeth, these tissues can be harvested postnatally from teeth extracted before the root formation and/or eruption of the tooth is complete [18, 19]. Stem cells of deciduous teeth contribute to the regeneration and maintenance of function of deciduous teeth of primary dentition. Since deciduous teeth are naturally exfoliated at different ages, the stem cells from human exfoliated deciduous teeth (SHED) are used for post-natal stem cell banking [22].

Isolation and characterisation of tooth-derived stem cells

The isolation procedure for tooth-derived cells starts with the collection of discarded extracted teeth in a suitable collection medium that contains antibiotics to eliminate microbial contamination, as well as enough nutrients to preserve the vitality of dental tissues present [23]. An appropriate cell culture facility is mandatory for the isolation of tooth-derived cells.

The isolation of cells can be done using two methods – i) tissue digestion method and ii) tissue explant culture method. In the tissue digestion method, digesting enzymes such as collagenase and dispase are used to disengage the cells from within the tissues and the ECM and these cells are collected by sieving followed by centrifugation. This technique may need a larger number of tissue samples to obtain an adequate cell population. In the tissue explant method, 1-2 mm fragments of dental tissues are cultured in regular cell culture media until cell outgrowths from the explant are obtained, which will be expanded further for experiments [22 - 24]. Figure 2 represents the isolation and characterisation steps of primary human periodontal ligament cells.

Characterisation of tooth-derived cells can be carried out using specific markers such as cementum membrane protein (CEMP) and Scleraxis for periodontal ligament cells. They exhibit properties similar to mesenchymal stem cells, such as plastic adherence or ability to adhere to a substrate, self-renewal by means of cell division and potential for multilineage differentiation. In addition, the tooth-derived stem cells exhibit common MSC markers such as CD 90, CD 105, CD 73 and STRO 1, and the intermediate filament protein Vimentin indicating the fibroblast nature of the cells. Routine microscopic evaluations can identify the fibroblast morphology and adherence to cell culture surfaces. Characterisation of the cells is invaluable to confirm the identity of the tooth-derived cells for further use in *in vitro* experiments [25]. Figure 2 is a schematic representation of the steps in explant culture and immunohistochemical charecterisation of the human peri-

Table 1: Characterization of tooth-derived cells – specific markers

Vimentin	Type III intermediate filament protein present in fibroblasts and mesenchymal cells.
STRO 1, CD 90 (Thy 1), CD 73 (ecto-5'-nucleotidase), CD 105 (Endoglin)	MSC markers. More than 90% expression of these markers suggests the mesenchymal stem cell nature of the isolated cells.
DMP 1 (Dentin Matrix Protein 1)	Non collagenous protein found in dental pulp and periodontal ligament.
Scleraxis	Ligament specific protein found in periodontal ligament cells
Cementum Membrane Protein (CEMP)	Cementum specific protein found in cementoblasts

odontal ligament cells grown for MSC markers CD 90, CD 105, CD 73 and STRO 1, and the intermediate filament protein Vimentin. The details of the specific markers for dental progenitor cells are given in table 1.

Differentiation of tooth-derived stem cells

Once characterised, the differentiation potential of the cells could be studied using specific culture media that can induce differentiation. The tooth-derived cells are known to

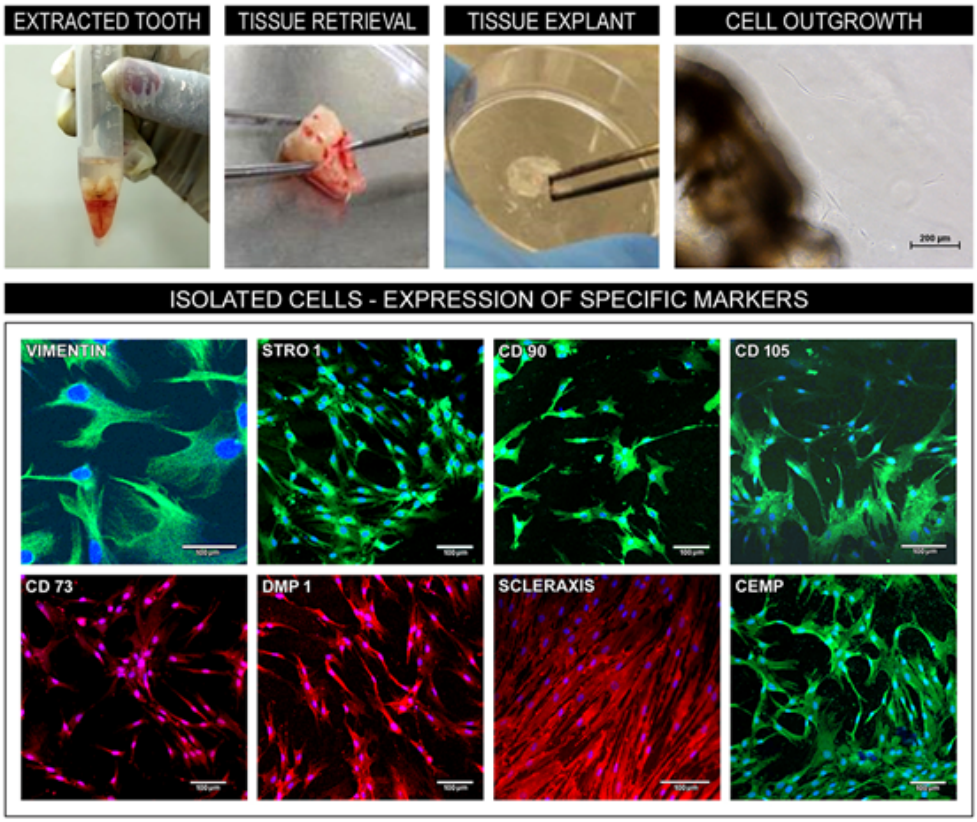

Fig 2: The steps of explant culture for the isolation of primary human periodontal ligament cells and their characterization are shown. The cells exhibit positive expression of the fibroblast marker (Vimentin), common MSC markers (STRO 1, CD 90, CD 73 and CD 105, DMP 1), ligament specific marker (Scleraxis) and cementum specific marker (CEMP)

differentiate *in vitro* into a multitude of lineages, including cardiac cells, neuronal cells, pancreatic islet cells, hepatocytes, retinal, corneal cells etc. The tooth-derived cells can differentiate into the respective hard tissue components *in vitro* and *in vivo*. The dental pulp stem cells can be induced into osteogenic, odontogenic, and dentinogenic lineages [26]. The apical papilla and the dental follicle are the tissues retained from the early embryonic development of the teeth and they can differentiate into adult dental pulp and periodontium respectively [19].

The tooth-derived cells may exhibit variations in their differentiation potentials, but similar histological responses and protein marker expressions are applicable for osteogenic (bone forming), cementogenic (formation of cementum, the hard tissue that covers tooth root), and odontogenic (dentin formation) differentiation since the differentiation to these three lineages depends on type 1 collagen deposition, and its mineralization at varying levels [26]. A list of the different tooth-derived cells, the corresponding source tissues and their differentiation potential is detailed in table 2.

Advanced techniques such as immunofluorescence staining and assays, PCR, western blot etc., have aided in the identification of tooth-derived cell responses to various mechanical, physicochemical, and biologic stimuli *in vitro* [24 - 26]. For example, the osteogenic differentiation of periodontal ligament cells and dental pulp cells can be histologically assessed using Alizarin red staining for calcium deposits, ICC-IF staining for osteogenic marker expression, alkaline phosphatase assay for enzyme estimation and/or PCR evaluation of osteogenic gene expressions.

Applications of tooth-derived cells

The tooth-derived cells are widely reported to differentiate and exhibit mineralization potential, which is an important indication towards osteogenic, odontogenic and cementogenic regeneration [1]. Embryologically, these tooth-derived cells are found to exhibit similar differentiation pattern during embryonic and post-natal tooth development. The mineralization pattern and the positive expression of specific marker proteins and genes, can be interpreted to understand how the dental pulp cells and apical papilla cells are able to differentiate to an odontogenic (dentin forming) lineage; and the periodontal ligament cells and dental follicle cells are able to differentiate to osteogenic (bone

Table 2: Properties of tooth-derived cells and their differentiation potential*

Source tissue	Tooth-derived cells	Differentiation potential
Adult Dental pulp	Dental pulp cells (DPCs), Dental Pulp Stem Cells (DPSCs)	*In vitro* : Odontogenic, osteogenic, adipogenic, chondrogenic, myogenic, angiogenic, neurogenic, hepatogenic, cardiomyocytes, pancreatic islet cells and immunomodulation. *In vivo* : Odontoblasts, dentin like structures.
Periodontal ligament	Periodontal Ligament cells (hPDLCs) Periodontal Ligament Stem Cells (hPDLSCs)	*In vitro* : Cementogenic, osteogenic, adipogenic, chondrogenic, myogenic, neurogenic, hepatogenic and immunomodulation. *In vivo* : Periodontal ligament like and/or cementoid tissues, osteoid like tissues.
Dental Apical Papilla	Stem Cells of Apical Papilla (SCAP)	*In vitro* : Odontogenic, osteogenic, adipogenic, chondrogenic, myogenic, angiogenic, neurogenic, hepatogenic and immunomodulation.
Dental Follicle	Dental Follicle Stem cells (DFSCs)	*In vitro* :Osteoblast, cementoblasts, adipocyte, chondrocyte, hepatic cells, neuronal cells.
Deciduous teeth tissues	Stem cells of human exfoliated deciduous teeth (SHED)	*In vitro* : Odontogenic, osteogenic, adipogenic, chondrogenic, myogenic, angiogenic, neurogenic, hepatogenic and immunomodulation.
Gingiva	Gingival Fibroblasts Gingival Stem Cells(GSCs)	*In vitro* : Osteogenic, adipogenic, chondrogenic, and immunomodulation.

* Compiled based on references [16 - 21]

forming) and cementogenic (cementum forming) lineages [26]. The cellular regenerative responses are subjected to alterations in their immediate surroundings (including the cell ECM) and are influenced by inflammation due to traumatic and microbial insults, ischemic conditions restricting blood flow to the tissues, and/or other mechanical, thermal, physicochemical, or biologic insults.

Utilising the toothderived cells for therapeutic applications is still in the exploratory stage due to various ethical and safety concerns arising from the laboratory handling and manipulations of these cells [27]. However, the *in vitro* differentiation potential of the tooth-derived cells and the various identification techniques makes them ideal candidate cells to study biological responses to various dental biomaterials, drugs, mediators etc., *in vitro* [28]. Utilising tooth-derived cells for cytocompatibility assessment will impart better clarity in understanding cellular responses to biomaterials used for dental applications. A representative image of human periodontal ligament cell morphology on different types of biomaterials is provided in figure 3.

In addition, tooth-derived cells can be used to evaluate tooth-specific cellular responses including differentiation to dentinogenic, odontogenic and cementogenic lineages, which is presumably not possible with unrelated cell lines or other primary cells. As described earlier, there exists various evaluation procedures such as histological staining, ICC-IF staining, enzyme assays, protein estimation procedures and gene expression studies using PCR, to understand cellular responses to regenerative dental biomaterials. In addition, specific spheroid culture methods, coculture methods, cell migration assays etc., can be utilized to study customised biological responses. A representative image of differentiation of human periodontal ligament cells and dental pulp cells on a bioactive biomaterial is given in figure 4.

All these evaluation parameters will hasten the biomaterial development process and help to reduce the time elapsed between bench to clinic translational approaches. The es-

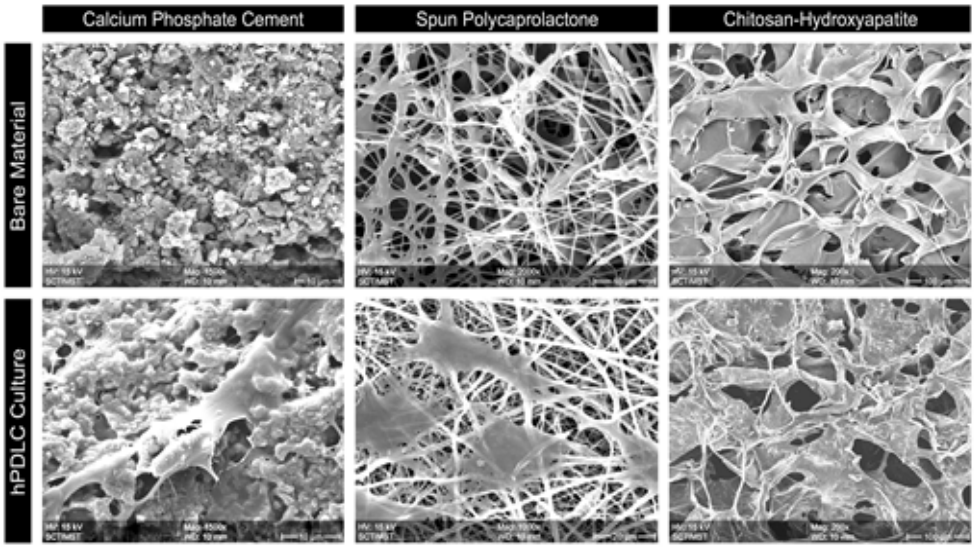

Fig 3: Representative images showing cell adhesion of human periodontal ligament cells (hPDLCs) on different types of biomaterials - hPDLCs on calcium Phosphate cement (CPC), electrospun PCL scaffold, and chitosan-hydroxyapatite scaffolds (CH-HA) showing morphology and adhesion of human periodontal ligament cells to different types biomaterial surfaces. The top row is biomaterial scaffolds without cells and bottom row with hPDLCs respectively. The close adaptation of tooth-derived cells to the potential biomaterials and maintenance of their cytoskeletal architecture within the biomaterials are indicative of possible host cell responses *in vivo*

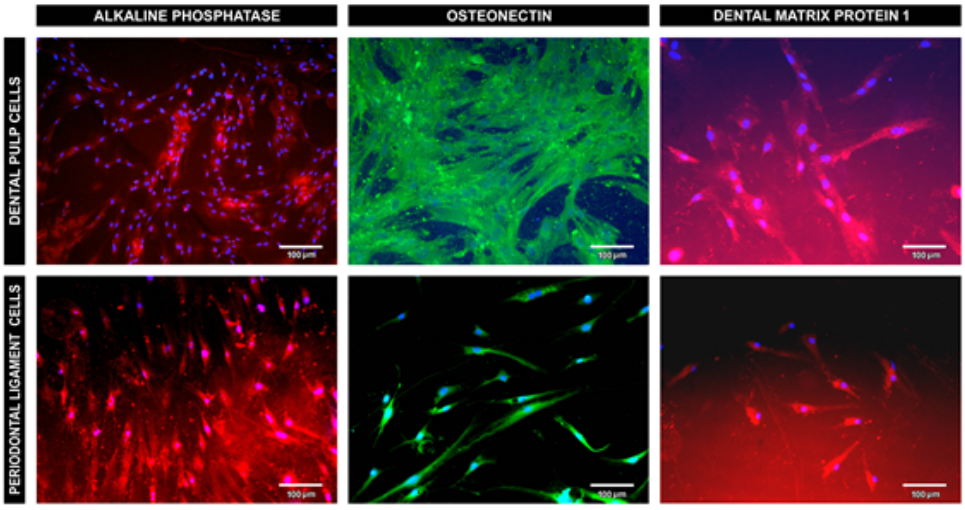

Fig 4: Demonstration of the differentiation of tooth-derived cells in the presence of biomaterial. Isolated periodontal ligament cells (hPDLCs) and human dental pulp cells (hDPCs) were cultured in the presence of a calcium sulfate based cement. Pictures show ICC-IF staining of osteo/odontogenic markers – Alkaline phosphatase (ALP), Osteonectin (ON) and Dentin Matrix Protein 1 (DMP 1). In both cases, positive expression of the markers is evident

tablishment of a standardised cell culture system can provide invaluable information on biomaterials/scaffolds for their use in regenerative dentistry. Once established, the differentiation potential of the cells can be utilised for the *in vitro* assessment of biomaterials, drugs, growth factors etc., which can be directly correlated to the cellular responses of host dental tissues.

Stem Cell Based Regenerative Approaches in Dentistry

Regenerative dentistry entered a new era with the advent of 'Tissue Engineering' techniques. Generally, these involve cells, scaffolds, and biological factors to facilitate the regeneration of damaged tissues. Regenerative Dentistry could be considered a simpler version of the conventional Tissue Engineering, as the dental tissues have the necessary stem cells in the tissues, which are relatively easy to access. Therefore, appropriate scaffolds, on implantation, can home the local cells and absorb factors which initiate regeneration.

Stem cell-based treatment approaches in dentistry are still in experimental stages with futuristic plans of cell-laden tissue-engineered scaffolds, scaffold-free cell delivery approaches, cell sheet delivery approaches etc. [29]. Some of these clinical trials have shown positive results with scope for further cell-based therapies in future. Hurdles on the ethical and technical fronts in achieving successful stem cell-based therapies must be the reason for the comparatively low number of clinical trials. No dental stem cell-based therapies are reported from India.

Endogenous treatment strategies

Understanding cellular regenerative responses by dental stem cells can help the development and optimization of novel regenerative treatment approaches [30]. One such treatment approach is the 'endogenous regenerative treatment', wherein the stem cells native to the defective host tissues are recruited and modulated towards regeneration through the prudent application of biologics, growth factors, biomaterials etc. [31]. This could be achieved in two ways – (i) Bring about the host cell homing and differentiation by making

use of inherent regenerative potential of the progenitor cells within the tissues, or (ii) Modify the host progenitor cell responses towards regeneration through extraneously supplied biomaterials, growth factors, mediators, and/or bioactive molecules that can mobilise and modulate the resident progenitor cells within the host tissues [32]. Endogenous regenerative approaches are gaining importance in facilitating the regeneration of dental tissues by modulating the responses of endogenous stem cells towards regeneration.

Biologics for endogenous regeneration

The best example of endogenous regenerative treatment approach is the regeneration achieved by natural means through the application of biologics such as PRP, PRF etc, derived from the patient's own blood and delivered to the site of the defect as a reservoir of growth factors and as a biological fibrin matrix simulating the ECM [33]. They provide the best matrix for the homing of host progenitor cells from the adjacent healthy tissues to induce differentiation and regeneration. However, autologous biologics necessitate minimally invasive procedures such as collection of blood, which may be of concern in patients compromised by bleeding disorders, anticoagulant therapies etc. Moreover, additional equipment and infrastructure are needed for the isolation processes. External manipulation of blood and derivatives are to be carried out, which are technique sensitive and often expensive.

Biofunctionalization for endogenous regeneration

Although biologics derived from a patient's own blood can predictably favour regeneration, the collection of blood through invasive procedures and techniques to isolate the biologics in a sterile environment may not be practical in every clinical scenario due to patient factors, technique sensitivity and/or cost. In such situations, artificially created biomimetic environments can offer an ECM-mimetic environment conducive to regeneration. One approach is the biofunctionalisation of scaffolds and biomaterials. Favourable cellular responses can be achieved through biomaterials based on synthetic ECM-mimetic matrices, biofunctionalised matrices, regenerative matrices etc. [34]. Biofunctionalisation of inert matrices using ECM-mimetic peptides can also modulate the host tissue environment towards regeneration [35]. The resident progenitor cells within the dental tissues form an important component for predictable regeneration. Synthetic biominerals, such as hydroxyapatite and biomatrices made of collagen, chitosan etc., are proven to induce the formation of bone and dental tissues [36]. The *in vitro* responses of human periodontal ligament cells to biofunctionalised electrospun PCL mat can be seen in Figure 4.

The current concept of tissue engineering with exogenous cells and mediators incorporated in a suitable scaffold is not practical in tooth regeneration because of the requirements of cell sourcing and preservation of cell viability within the scaffold. Alternatively, endogenous homing of resident cells like odontoblasts, fibroblasts (of dental pulp and periodontium), cementoblasts, osteoblasts and stem/progenitor cells could be promoted to aid regeneration, with the use of appropriate scaffolds. The blood clot formed at the site of injury can act as a natural scaffold, which can release the innate growth factors to recruit circulating and local stem cells and progenitors for regenerating new tissue, through complex signalling cascades [32].

Biomaterials and Scaffolds for Regeneration

Biomaterials have been in use as bioactive restorative materials (that promote remineralisation), dental pulp capping agents, materials for apexification and revascularisation, GTR barrier membranes, and bioactive bone grafts. The biomaterials used in regenerative dentistry can be divided into three categories - (i) Natural

biomaterials like collagen, chitosan, decellularized tissue matrices, demineralised bone etc. ; (ii) Synthetic polymeric materials like PLA, PLGA, etc., and (iii) Synthetic inorganic materials like calcium phosphates, hydroxyapatite, bioglass etc. [34 - 37]. A selected list of commercially available biomaterials-based products for regeneration applications is given in Table 3.

Conventional biomaterials in dentistry

Biomaterials have been utilised as adjuvants or scaffolds in harnessing the inherent regenerative potential of dental tissues. Calcium hydroxide is probably the earliest biomaterial used for regenerative purposes, especially for reinstating damaged dental pulp. The action of calcium hydroxide is two-pronged - (i) Establishing an alkaline environment at the site of application (pH in the range 10-12), thereby creating an anti-microbial local environment, and (ii) Providing calcium ions for initiating an osteogenic response. These actions together promote mineralised tissue formation *in vivo*, which is predominantly a reparative response [38]. The poor handling characteristics of calcium hydroxide powder led to the invention of hydraulic self-setting cement as an alternative material. Products such as mineral trioxide aggregate (MTA) became popular, yet they were slow resorbing and slow setting. Calcium phosphate formulations have been tried, which provide calcium as well as phosphate ions to the injured pulp tissue to promote predictable regeneration. Some of these formulations were incorporated with antibiotics as well [39, 40].

Periodontal treatment was mainly centred on the guided tissue regeneration (GTR) technique (as described in section 2.2.1), wherein several kinds of membranes made of different materials were used. Today, resorbable membranes have replaced the non-resorbable membranes used earlier. Among them, collagen-based GTR membranes became the standard product [41]. Modern-day GTR membranes are bioactive materials with the capability of controlled delivery of biomolecules, antimicrobials, drugs etc., to promote periodontal regeneration [42]. Though the selective cell repopulation worked for tissue loss in the early stages of periodontitis, significant alveolar bone loss due to periodontitis required grafting of the bony pockets formed. In this procedure, bone grafts as chips or granules in small sizes are filled in the defect so as to make them integrate with the natural remodelling of the alveolar bone defect [43]. Natural bone allografts (decalcified freeze-dried bone), xenografts (processed bovine bone) and bone derivatives (collagen, growth factors and proteins) were used for bone grafting by virtue of their biological potency to enhance bone remodelling. Demineralised bone and several collagen-based products are available commercially for this purpose [44]. Enamel matrix derivatives (EMD, the proteins isolated and purified from the developing porcine teeth) have also been tried, with favourable outcomes [45]. The risks of cross-species pathogen transmissions and immune responses shifted the focus to synthetic alloplastic graft materials comprising of bioceramics (or biocompatible inorganic compounds).

Bioactive alloplastic materials

Fine porous ceramic granules of hydroxyapatite (HA), the basic mineral of bone, and silica-based glassy materials such as bioactive glasses have been explored extensively for bone grafting applications. Calcium phosphate ceramics are preferred because they show *in vivo* resorption [46]. The performance of synthetic bioceramic grafts is mainly dictated by 'bioactivity' (ability to establish chemical attachment with hard tissue), osteoconductivity (capacity to allow bone tissue to grow over the surface) and osteoinductivity (ability to induce bone formation), which are desirable biological properties for alloplastic materials [47]. Materials with bioactivity that can be grafted to bone defect areas can induce new bone formation and can be replaced with natural bone as they get resorbed into the body. The earliest in this category is calcium sulphate (in hemihydrate form, better known as 'plaster of Paris'). Being inexpensive, biocompatible,

mouldable and resorbable cement, it has been used for guided tissue regeneration (GTR) in periodontal repair. Calcium sulfate modified with calcium phosphates can act as resorbable scaffolds, on top of which natural bone could be built up [48]. HA is osteoconductive in nature but acts as a slow resorbing scaffold. Tricalcium phosphate (â-TCP) also is biocompatible and osteoconductive, but on implantation, it shows faster resorption. The resorption can be tuned to the clinical need by designing HA-TCP 'biphasic' compositions [46].

Enamel, despite being hard, is prone to demineralisation in the acidic conditions of the oral environment. Mechanical debridement during mastication and crown fracture also lead to enamel loss. Unlike the periodontal ligament and pulp-dentin complex, the formative cells of dental enamel (the ameloblasts) are permanently lost during tooth eruption to the oral cavity, and hence it does not get regenerated. However, remineralisation is possible if a local environment rich in calcium ions is created. Calcium-enriched and hydroxyapatite-containing dentifrices (toothpaste) have been developed for this purpose. The use of such dentifrices was observed to lead to enamel remineralisation with improved acid resistance by forming a protecting layer on the enamel surface but also improve their periodontal health [49].

Novel biomaterial-based approaches for dental tissue regeneration

The biomaterials for tissue regeneration have evolved through three generations – (i) Inert materials with no interaction with biological tissues, (ii) Bioactive materials eliciting controlled positive tissue responses, and (iii) Materials and structures having the potential to stimulate and modulate cellular and molecular events towards regeneration. The developments in the area of Biomaterials in the recent past has led to a better understanding of material-tissue interactions and lead to the development of newer strategies for achieving regeneration of dental tissues.

Bone, dentin and cementum could be considered as composites made up of an organic type 1 collagenous matrix with hydroxyapatite nano-crystals in a hierarchical arrangement. This specific structure endows it with unique mechanical properties, like the low stiffness combined with high fracture toughness to resist tensile and compressive forces [9,10]. Understanding the functionality and complexity of biological systems and knowledge of basic biological principles of collagen deposition and biomineralization processes is essential to design appropriate biomaterials for the regeneration of alveolar bone, dentin, and cementum [37]. Ideally, for dental pulp regeneration to be complete, the tissue formed should be vascularized, innervated, with dentinal walls lined by newly differentiated odontoblasts that are capable of continued dentin deposition in its original tubular pattern onto the existing dentin matrix [11]. This can be enhanced by proper biomaterials that can bring about regeneration and are able to resorb in unison with the new tissue formation [4].

A notable class in the new generation materials in synthetic bone graft family is the self-setting calcium phosphate cements (CPCs) which are aqueous based and do not require any initiators. They are provided as powder-liquid combination, the mixing of which in the prescribed ratio gives bone mineral phase of calcium phosphate. CPCs are mouldable and suggested for different regenerative applications dental pulp capping, apexification and periodontal defect repair, with promising results in animal studies and clinical trials [50]. Similar regenerative responses are also obtained when CPCs were used for the non-surgical treatment and healing of periradicular lesions associated with extension of infections of dental pulp [51]. These studies have led to the development of combinatorial biomaterials with improved handling properties as well as biologic properties.

A co-ordinated deposition of newly formed cementum and alveolar bone with collagen fibres interspaced between these two hard tissue components (by means of healthy Sharpey's fibres) is needed to achieve periodontal ligament regeneration. Collagen barrier

Table 3: Commercially available biomaterials based products for regeneration applications

Treatment	Material composition	Commercial brands
Vital Pulp Therapies, Revascularization, Endodontic Repair, and regenerative therapies.	Calcium Hydroxide	Dycal (Dentsply), Apexit & Apexcal (Ivoclar), Cal-Plus (Prevest), Ultracal (Ultradent), Sealapex (Sybron Endo) etc.
	Mineral Trioxide Aggregate (MTA)	ProRoot MTA (Dentsply), MTA Angelus (Angelus), MTA Plus Aqua (Prevest Denpro) etc.
	Calcium Silicate and Bioceramics.	CeraSeal B (Meta Biomed), Bio-C Sealer (Angelus), Dia-Root BioAggregate (Diadent), Biodentine (Septodont),
Alveolar bone grafts for GTR/GBR	Xenogenic and allogenic bone grafts	Bio-Oss (Geistlich), Cerabone (Straumann Botiss), Osteograf (Dentsply), Osseograft DMBM (Advanced Biotech), etc.
Synthetic Alveolar Bone grafts for GTR/GBR	Hydroxyapatite and Bioglass	Maxresorb (Straumann Botiss), Novabone Bone graft (Calcium Phospho-silicates), Perioglas (Novabone), BoneGraft HA Nano (B-Ostin), etc.
	Synthetic Calcium phosphates	RTR-Bone Graft Material [ß TCP granules] (Septodont), DM-Bone [Biphasic Calcium Phosphate] (MetaBiomed), Ostoden Bone Graft (Ammdent), B-OstIn Bone Graft TCP (Basic HealthCare), etc.
	Calcium sulfates	GraftSet® (Salvin), CapSet (Trycare Ltd), Calcium Sulfate Bone Graft Binder (Calmatrix), OsseoMold [Calcium sulfate-DMBM] (Advance biotech), etc.
Barrier membranes for GTR/GBR	Collagen	Colo Gide (Cologenesis Healthcare), Healiguide (Advanced Biotech), Bio-Gide (Geistlich), Biocollagen (Bioteck), etc.
	Poly Lactic Acid	Epiguide PLS (iRES.dental)
	Poly Lactide Poly Glycolide Co-polymers	BioMesh S (Biomesh), Cytoflex Resorb (Cytoflex), etc.

membranes, applied alone or combined with alveolar grafts, are used in GTR/GBR procedures to achieve periodontal regeneration. Calcium phosphate cements, hydroxyapatite and bioactive glass in fine granular form have become materials of choice as bone grafts in alveolar defect management. Predictable regeneration of alveolar bone and periodontal ligament attachment apparatus, are reported these materials [46]. Calcium phosphate cements, being mouldable, osteoconductive and bioresorbable, are suggested to be used as 'barrier-grafts' for alveolar bone regeneration applications [46]. Another cementing material identified for bone grafting is gypsum cement, which is chemically calcium sulfate dihydrate. The hemihydrate form (known as plaster of Paris) will readily undergo hydration setting in presence of water and retain the moulded shape. Thus high pure Gypsum cement could be used as a bioresorbable and osteotransductive graft material. The osteogenic properties and bioresorption characteristics of Calcium sulfate Cements (CSCs) have been established in human clinical trials. Other formulations of CSCs with calcium phosphates were tried to improve the biological activity and to tune the resorption pattern to suit regenerative dental applications [48]. Another attraction of the CSC cements is that most of the antibiotics could be safely incorporated for local delivery to manage infections [46, 48].

These modifications of the basic formulation of calcium sulfate cements resulted in affordable regenerative dental cements with promising *in vitro* and *in vivo* results [48,52]. The preclinical evaluation revealed the novel combinatorial calcium sulfate-phosphate combination cements resorbed in the same pace as the new bone formation, which is known as osteotransductivity [48]. It will further help in faster healing and gain in strength and can be advocated for its use as a resorbing 'barrier graft' for alveolar bone re-

generation [52].

Combinatorial approaches using different types of biomaterials like natural and synthetic polymers, organic-inorganic biocomposites, biomaterials enriched with bioactive molecules etc., can enhance the regenerative potential of biomaterials. Current advances in material science including electrospinning, 3D bioprinting, *in situ* hybridization of inorganic salts such as calcium phosphates to natural or synthetic hydrogels, incorporation of growth factors and mediators into biologic matrices, etc. can elevate the existing treatment strategies with predictable results. Such matrices can be laden with growth factors, biomolecules, small bioactive peptides, nano-calcium formulations or anti-microbial agents [34-36]. Biomaterials can act as scaffolds or matrices to provide three dimensional supports for cells and promote selective repopulation of local progenitor cells through migration and cell adhesion followed by differentiation [30 - 36].

A host of biomaterials based products are now commercially available for regeneration applications. A limited list of the commercial brands used for dental treatment, and their material composition are compiled in table 3.

Biomaterial based scaffolds for regeneration

The term 'scaffold' is a tissue engineering concept, depicting a biologically appropriate matrix created endogenously or a biomaterial structure fabricated extraneously, designed to promote host cell adhesion and differentiation. Scaffolding technique aims to mimic the host extracellular milieu that is most conducive for regeneration. Currently, more attention is being given to achieve biological scaffolding as much as possible, by using appropriate biologics such as PRP, PRF, fibrin glue etc. [30 - 33]. Biomaterials are being constructed into intricate ECM-mimetic scaffolds through advanced microfabrication techniques such as 3D bioprinting, electrospinning etc., to develop customized multiphasic scaffolds for dental regeneration [36]. These scaffolds promote regeneration through the response of host progenitor cells such as the dental pulp cells and the periodontal ligament cells in response to the release of bioactive molecules, growth factors, mediators, and/or drugs. Regeneration is achieved through the cellular and molecular responses to the biomaterials, wherein the biomaterial structure act as supporting matrix or bioactive scaffold [38 - 40]. This modulation of host progenitor cells is the most recent development in biomaterial based regenerative approaches in dentistry [1].

On summarizing the tissue regeneration studies, biomaterial based regeneration can be divided into:

i) Regeneration of dentin by the formation of organized secondary dentin formation, or deposition of reactionary or reparative dentin,

ii) Regeneration of whole or part of dental pulp to preserve the vitality of the tooth,

iii) Regeneration of part or whole of periodontium involving cementum, alveolar bone and periodontal ligament,

iv) Remineralization of dentin and enamel mimicking the original tissue architecture, and

v) De novo regeneration of dental pulp like tissue within the dental pulp space from the apical papilla and/or periodontal ligament cells from periradicular area.

Future directions

Regenerative dentistry relies on the successful modulation of inherent regenerative responses of host progenitor cells within and around the dental tissues. These episodes of regeneration observed in individual cases when systematically studied to develop various regenerative treatment approaches of dental pulp and periodontium. What emerged as an ambitious concept in the last quarter of the past century, had evolved into successful treatment approaches with predictable regeneration of dental tissues. Regenerative den-

tistry is progressing to new arenas with the availability of better biomaterials, better knowledge on cellular responses and better methods for infection control. Treatment strategies such as vital pulp therapies, revascularization/ revitalization procedures, and GTR/GBR procedures for the regeneration of dental pulp and periodontium, are now generating better outcomes, thereby strengthening the regenerative concept. Tissue engineering strategies have complimented regenerative dentistry to bring together biomaterials, endogenous cells and mediators to enhance the outcomes.

Biomaterials development and optimization have significant roles in tissue engineering and regenerative applications in medicine and dentistry. The barrier membranes for periodontal regeneration have evolved from inert membranes that required surgical removal, to resorbable and bioactive membranes capable of enhancing cell homing and differentiation. Multiphasic and functionally graded scaffolds, injectable hydrogels and self-setting inorganic cements are being explored in periodontal regeneration. Functional grading of GTR materials give tuned resorption and enhanced tissue growth. Injectable, self-setting viscous cements can conformally fill alveolar bone defects, and promote bone and soft tissue regeneration. Injectable hydrogels are also investigated for regeneration of dental pulp in vital pulp therapies.

The rapid evolution of novel biomaterials necessitates a dependable biologic screening platform that can simulate the host cell responses *in vitro*. Rapid screening that allows modifications and optimization of biomaterials is possible if standardized *in vitro* cell culture systems using appropriate host cells are developed and made routinely available. The tooth-derived cells offer an easily accessible, affordable and simulative cell source for the rapid screening, which can provide useful information on biological responses *in vitro*. The novel biomaterials that emerged during the past decades (mainly bioceramics and cements for hard tissue regeneration) possess the capabilities of bioactivity, immunomodulation and infection control and their *in vitro* and *in vivo* responses are repeatable and predictable.

The restoration of the three-dimensional structural and functional architecture of the periodontium still remains a challenge for biomaterial-based regeneration approach. Similarly, predictable dental pulp regeneration with formation of aligned tubular dentin containing healthy odontoblastic processes is also a challenge in regenerative dentistry. Total tooth regeneration is still in hypothetical stage with limited number of studies. These challenges could be circumvented through the introduction of bioactive materials which have the capacity of 'directed regeneration' facilitating the tunable and appropriate response *in vivo*. An efficient *in vitro* cell culture system with tissue specific tooth-derived stem cells, can help in expediting the *in vitro* evaluation process of such materials. Scaffolds corresponding to the local tissue architecture and with biological cues can host the stem cells *in vivo* and lead to complete structural and functional regeneration.

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