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Regenerative endodontics: Cell-based versus cell-free approach

Jittranan Kaewprag[1] , Nisarat Ruangsawasdi[2]

[1] *Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University*
[2] *Department of Pharmacology, Faculty of Dentistry, Mahidol University*

In regenerative endodontics, the cell-based and cell-free approach in immature permanent teeth is a novel root canal treatment that attempts to regenerate a functional pulp rather than a mere prosthetic material in the root canal system. However, the outcomes of this treatment are still uncertain as they depend on various factors. Accordingly, there is a need for better understanding the biology behind pulp regeneration as well as an improving tissue ingrowth into the pulp space. This review will focus on the update horizon of alternative approaches in regenerative endodontic and provide the insight into the account of pulp tissue engineering.

Key words: cell-based approach, cell-free approach, pulp regeneration, regenerative endodontics.

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Introduction

Root canal treatment of immature teeth with incomplete root formation may potentially require removing the diseased pulp (pulpectomy) and apexification. Recently, regenerative endodontics has emerged as an alternative treatment for immature teeth with infected/necrotic dental pulps. The objective of this treatment is to regenerate a functional pulp that maintains pulp-dentin interactions. [1] The root and dentin of immature teeth do not continue to develop after conventional treatments, whereas the completion of dentin growth accompanied by apical closure could be enabled by regenerative endodontics.

A plethora of potential treatment strategies have been suggested after the discovery of various stem cell sources in the oral cavity, especially the apical papilla tissue, which surrounds the root apex of immature teeth and contains mesenchymal stem cells. [2] Accordingly, clinicians have attempted to induce the apical papilla to bleed and thus infiltrate the previously cleaned and disinfected pulp space with pluripotent cells. [3] The outcomes, however, are still unpredictable with a substantial number of reported failures. [4] Although some researchers support stem cell utilization for tissue engineering [5], clinicians hitherto omit this application to patients due to difficulties and tissue loss during the harvest of potential stem cells, as well as the uncertainty about the effects to the host. [6] Therefore, the stem cell-based treatment mostly remains at the laboratory level.

In the near future, the so-called cell-free approach appears to be the more reliable and more foreseeable option. This concept, which has

Correspondence author: Nisarat Ruangsawasdi
Department of Pharmacology Faculty of Dentistry, Mahidol University
6 Yothi Road, Ratchathewi, Bangkok 10400
Tel: +66-2-200-783-3 ext.16 Fax: +66-2-200-783-4 Email: nisarat.rua@mahidol.ac.th
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been coined cell homing, is to recruit host stem cells into a specified area using molecular cues to appropriately direct cell differentiation. Given the evidence of various stem cell sources in the oral cavity, this approach is easier translated into clinical practice. There is no need for stem cell harvesting or any certification. Only scaffold materials and growth signaling molecules are required to initiate host stem cell migration to the pulp space. [6]

Although cell homing has a higher chance for clinical translation, there are still several hurdles to overcome. The optimal scaffold is still unknown and the signal cues necessary for pulp regeneration are also ambiguous. Moreover, pulp tissue is a complex structure containing four different regions of soft connective tissue. It needs to integrate with the hard mineralized dentin and is composed of a variety of cell types. Regeneration of new functional pulp tissue might require the optimal combination of scaffold and signal cues, which can attract stem cells that should differentiate into a specific cell type at the appropriate location. Various aspects should be investigated so that we can apply advanced biotechnology in regenerative endodontics and find opportunities for clinical applications.

Trend of Pulp Regeneration in endodontic treatment

Endodontic treatment is needed when the defense process within the pulp tissue fails to protect against invading bacteria. As the protective system collapses, inflammation spreads throughout the whole pulp space, finally resulting in tissue necrosis. The main goal of root canal treatment is thus to remove the infected pulp and subsequently seal the tooth to prevent further ingress of oral bacteria and the thus-resulting inflammatory reactions in the tissues surrounding the root apex.

The contemporary treatment is not always the best option, particularly in regard to contemporary ways to fill cleaned and disinfected root canal systems. Root canal fillings with gutta-percha and a sealer certainly suit the fully developed permanent tooth, whereas failures occur frequently when using this method to treat the immature tooth. [7-9] The poor mechanical characteristics of young permanent teeth do not only cause trouble during the endodontic procedures, but also affect sustainability after the treatment. [10]

A novel attempt has emerged over the past decade to regenerate a functional pulp in formerly infected immature teeth, i.e. immature teeth with apical periodontitis. A classical study by Torneck et al showed that connective tissue could be formed in the emptied space of cylindrical tubes in rodents. Although the growth was less extensive when the tube's lumen was too long or too narrow, the study inspired the possibility of tissue in-growth into the limited area of the pulp space. [11] The concept was later transferred to human permanent teeth whereupon bleeding was induced, allowing fibrous connective tissue, as well as in some instances cementum-like tissue, to also form in the root canal. [12] However, these early reports were on mature teeth with fully formed roots. Tissue ingrowth only occurred in teeth containing a vital pulp stump, not counterparts that were fully necrotized and had a periapical lesion. Nevertheless, these studies together with the identification of stem cells in the apical papilla tissue supported the idea to induce pulp regeneration in immature permanent teeth instead of restoring them with synthetic materials. [2, 13]

The first successful case, which was perhaps performed accidentally, was reported in 2001 in which, after an antibiotic paste was left in an infected immature premolar for an extended period. The radiographic image of the treated tooth revealed an increase in root length and thickness as well as a reduction in pulp space. [14] These outcomes triggered the application of a new therapeutic concept to achieve successful pulp revascularization, which is achieved by intentional bleeding into the formerly disinfected root canal. [15] From the moment that this guideline was proposed

to the endodontic field, a multitude of case reports and case series have gradually been published. [16]

More recent studies, however, reported negative outcomes [17, 18] , especially when the infection reached beyond the root canal. These incidents may have occurred because the apical papilla and its potential stem cells were already destroyed. Therefore, the major challenge in regenerative endodontics is pulp regeneration in the absence of a vital apical papilla, which is also the case for mature teeth. Accordingly, it is questionable whether the invading cells following the evoked-bleeding step would have the same potency as stem cells from the apical papilla to regenerate pulp. Stem cell-based approaches have thus been proposed to overcome problems of predetermination of the cells. However, a recent study revealed mesenchymal stem cell markers present on recruited cells following intra canal bleeding, not only in immature tooth [19] , but also in mature teeth. [20] These findings strongly suggest differentiation potency of the migrated cells for the reconstruction of dental pulp even without the application of stem cells.

Despite reports on failure of regenerative endodontics [5] , the treatment has significantly improved clinical results in immature permanent teeth. The success rates of these procedures are ranging from 79%-90% with increase in root length and root width as compare to apexification. [16, 21, 22]

Cell-based approach

The topic of pulp regeneration has received a lot of attention from both researchers and clinicians looking to improve the concept. The focus is on how to assemble the triad of tissue engineering components: stem cells, scaffold and growth factors. All components are mandatory in the course of tissue engineering, but the method may differ regarding the derivation of the stem cells. Accordingly, some groups believe that the action of autologous stem cells may be insufficient for positive long-term results and propose that cells with the potency to form the dental pulp tissue should be applied. [23, 24]

Most protocols of cell therapy suggested to date have combined isolated stem cells with an adequate scaffold under appropriate morphogenic signaling. *In vivo* cell transplantation studies have shown full regeneration of dental pulp-like tissue in the root canal including the deposition of new tubular dentin. [5, 25, 26] In general, the target stem cells are from one of the following niches: dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), stem cells of apical papilla (SCAP), bone marrow mesenchymal stem cells (BMSCs) and adipose-derived stem cells (ADSCs). Their manipulation normally requires retrieval and *ex vivo* expansion in the laboratory prior to use. Therefore, a protocol should follow the good manufacturing practice (GMP) guidelines, however excessive costs will make it difficult to implement in clinics. [27] Nakashima et al has been tried to prove the safety, efficacy and feasibility of stem cell transplantation in the root canal over a decade. [24, 28-30] Their success is now in the stage of first clinical trial, which likely shows a promising result. The DPSCs with GMP were loaded into the disinfected root canal of the mature permanent teeth together with the collagen scaffold. Around three months later, the vitality of dental pulp could be regained and there was no report on the significant adverse events. Only the evidences of no electric pulp response with one patient and the widening of periodontal ligament space with another patient occurred due to microleakage of the restoration. [31]

Cell-free approach

In contrast, the cell-free approach has shown the most promise for clinical translation. According to reported cases with induced bleeding, the generation of a blood clot forms a scaffold for apical papilla cells to home, while

other growth factors are released from platelets during the clotting cascade and are also present at the pulp-dentin interface. [32] Although the main components necessary for tissue engineering appear to be fulfilled already in this current clinical concept, it is still somewhat uncontrollable and unpredictable. Side effects such as tooth discoloration are frequent, and continued root development is not achieved routinely. Time to clot formation is random and in some cases the blood clot is insufficient. [4] The histological evidences showing a complete pulp-dentin regeneration are still questionable. The systematic review of histological studies assessing regenerative endodontic treatment in animal teeth reported that only 2% of the experimental teeth showing dentin-like hard tissue formation, whereas, 53% and 5% of the experimental teeth exhibited the deposition of cementum-like and bone-like hard tissue in the dentinal wall respectively. [33] Moreover, the revascularized immature teeth that had to be extracted for reasons unrelated to the procedure also showed that the tissue that forms in the root canal space is not pulp, but rather of periodontal origin. [34]

Attempts to improve the cell homing concept thus should focus on how to enhance the potency of the scaffold, also by determining necessary signaling molecules. [3] The scaffold should mimic the structure of extracellular matrix of the pulp and it should have the cells friendly properties. Accordingly, the scaffold characteristics are accounted for a soft form matrix, capable of shape modification and degradable over the time. Previous study showed that the platelet rich plasma (PRP) could be used in cases with blood clotting insufficiency and provided the clinical success such as continued root growth and positive to electric pulp test. The PRP in such study functioned as the bioactive scaffold that could provide the release of growth factors from platelet and plasma components while it could maintain the cell residing properties. [35, 36] However, their limitations are the difficulty in preparation and drawing blood from the patients. [37] Hydrogel is the scaffold of choice which has been revealed in many studies. [38-41] There are many types of hydrogel, mainly divided into natural and synthetic hydrogel. However, the study by Galler et al revealed that the synthetic hydrogel unlikely produced a better cell response and fibrin hydrogel possibly become the chosen scaffold. [42] Our *in vivo* results also demonstrated an improvement in cell differentiation and tissue ingrowth when using the fibrin hydrogel loaded into the immature permanent tooth. [43, 44] The use of fibrin hydrogel allowed host cells to migrate further deep in the root canal and then differentiate to form a pulp-like tissue. Likewise, German et.al suggested the use of fibrin hydrogel for delivering stem cells in regenerative endodontic because it can be easily modified to various fibrin and thrombin formulation for different purposes in order to control cell behavior. [39] In retrospect, the fibrin hydrogel and the hydrogel derived from blood are more prone to be the scaffold used in regenerative endodontic since it guarantees the regenerative evidences both *in vitro* and *in vivo* result, as well as, in clinics.

Key success in regenerative endodontics

Apart from the strategies divided by cell usage, other aspects of the treatment protocol are under scrutiny in regenerative endodontics. There is evidence that root canal irrigants and medicament may have adverse effects on new tissue formation. [45] Disinfectants like sodium hypochlorite and antibiotic root canal pastes can harm stem cells and impede revascularization in the root canal. [45-47] In contrast, bio-compatible chelating agents such as Ethyldiaminetetraacetic acid (EDTA) have been suggested to condition the dentine prior to cell relocation. EDTA-mediated effects include liberation of growth factors such as

transforming growth factors-beta 1 (TGF-β1), bone morphogenic proteins-2 (BMP-2) and fibroblast growth factor-2 (FGF-2) from the dentinal wall. EDTA thus promotes collagen-based dentin matrix directly in contact with the cells and it helps to reveal the appropriate qualitative and quantitative growth factors on the exposed collagen matrix. [48-50] In our study, the treatment of dentinal surface with EDTA concurrently showed greater cell integration and differentiation *in vivo* compared to non-EDTA irrigation (unpublished data). We rinsed the dentinal surface of the immature premolars with or without EDTA prior to loading the fibrin hydrogel into the pulp chamber and then embedding in the dorsal area of rat. Six weeks later, intra pulp space showed not only cells extending their cytoplasmic process into the dentinal tubules at dentine interface but also in the root canal revealed the differentiated cells rather than inflammatory cells which was commonly found in the non-EDTA treated dentine (Figure 1). Likewise, a calcium hydroxide suspension has been recommended to replace the use of antimicrobial for intra root canal medication because the concentration of antibiotic over than 1 mg/ml seemed to be cytotoxic to the stem cells derived from apical papilla tissue. [46] On top of that, minocycline combined in triple antibiotic paste might impede revascularization process regarding its anti-angiogenesis properties. [51] These clinical procedures, employed before bleeding activation, appear to be important regarding their impact on pulp tissue regeneration. However, they have not yet been standardized.

Conclusion

In summary, there are still further studies necessary to correct and improve the concept of the cell-based approach in regenerative endodontics before consistent clinical results can be reached. Meanwhile, the optimal scaffolds should be defined and the stem cell niches had better be approved, especially in infected root canal, for the possibility of cell-free approaches in clinics.

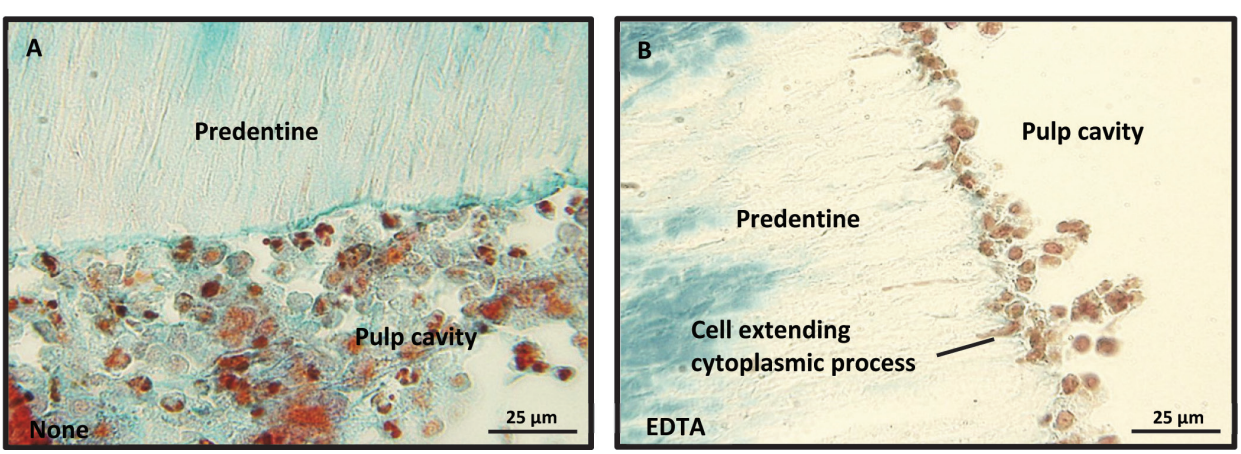

Figure 1 The effects of Ethyldiaminetetraacetic acid (EDTA) irrigation in the fibrin hydrogel loaded teeth that were transplanted in dorsal area of the rats. Root canal treated with 5% sodium hypochlorite alone (A) showed inflammatory cell residing and no cell-dentine integration. In contrast, the EDTA treatment (B) improved not only migrated cells but also cell-dentine integration and cell morphology at dentine interface.

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