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A Review of New Developments in Tissue Engineering Therapy for Periodontitis

Taka Nakahara, DDS, PhD

Section of Developmental and Regenerative Dentistry, School of Life Dentistry at Tokyo, The Nippon Dental University, 1-9-20 Fujimi, Chiyoda-ku, Tokyo 102-8159, Japan

Periodontitis is a highly prevalent chronic inflammatory disease and has been linked to systemic diseases, such as diabetes, cardiovascular disease, and respiratory diseases [1]. If untreated, this disorder can destroy the tissues that support the teeth (that is, the alveolar bone, periodontal ligament, cementum, and gingival tissue), which eventually leads to tooth loss. According to a survey performed in 1999 [2], roughly 50% of Japanese individuals in their 30s had lost at least one tooth, and the number of teeth lost per person increased sharply after the age of 40. Furthermore, more than 50% of Japanese people aged 85 years or more were found to be completely edentulous—that is, they had none of their 28 original teeth. Scaling and root planing are effective methods for preventing the progression of periodontitis in most cases. However, although these conventional treatments can eliminate the causes of periodontitis, they are unable to regenerate lost tissues, such as the alveolar bone, cementum, and periodontal ligament. In Japan, it is known that more than 80% of adults aged over 30 years have periodontal diseases, including gingivitis and periodontitis [2]. Thus, an urgent need exists to establish a new therapeutic method for this disease.

In 1993, Langer and colleagues [3] proposed tissue engineering as a possible technique for regenerating lost tissue, and the restoration of various human tissues and organs is starting to become a reality. The concept of tissue engineering was introduced originally to address the chronic shortage of donated organs. This approach reconstructs natural target tissue by combining three elements: a scaffold or matrix, signaling molecules (for example, growth and differentiation factors and genes), and cells. Current approaches

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E-mail address: t.nakahara@tky.ndu.ac.jp

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to tissue engineering can be divided roughly into two main types: ex vivo and in vivo (Fig. 1). In the former, the target tissue is created in a laboratory by culturing cells on biodegradable scaffolds in the presence of specific trophic factors before their transplantation into the body [4,5]. In the latter approach, the three elements mentioned above are placed into a tissue defect "in situ," and the tissue is restored by maximizing the natural healing capacity of the body by creating a local environment that is favorable for regeneration [6–8].

This article reviews tissue regeneration techniques, with an emphasis on the restoration of periodontal tissues, and discusses possible future directions based on recent technologic advances.

Tissue engineering for periodontal tissue regeneration

Protein-based approaches

Growth and differentiation factors can regulate the adhesion, migration, proliferation, and differentiation of various types of cell by binding to appropriate receptors. Recent advances in genetic engineering technology have made it possible to obtain large quantities of human recombinant proteins. The use of growth and differentiation factors is the most popular tissue engineering approach for regenerating periodontal tissues. So far, several growth factors—including transforming growth factor-β (TGF-β) superfamily members, such as bone morphogenetic protein-2 (BMP-2), BMP-6, BMP-7, BMP-12, TGF-β, basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF)—have been used to regenerate periodontal tissues [9–16]. Moreover, studies are underway currently to test the clinical potential of some of these factors [15,16].

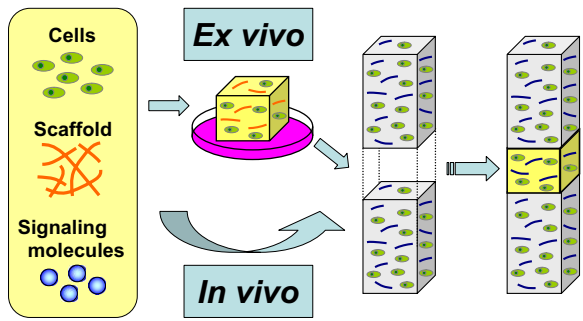

Fig. 1. Schematic representation of two major approaches to tissue engineering. First technique is to create tissue or organs in culture room by combining three elements (scaffold or matrix, signaling molecules, and cells) before transplanting tissue-engineered organ into patients (ex vivo approach). Second technique is to induce intrinsic healing activity at site of tissue defect using these three elements (in vivo approach).

Platelet-rich plasma (PRP) contains several platelet-released growth factors, including PDGF and TGF-β. PRP therefore represents an autologous growth factor cocktail that can be harvested from patients with minimal invasiveness and used for applications in oral and maxillofacial surgery [17–21]. PRP stimulates the proliferation of human osteogenic cells [22,23] and periodontal ligament cells [24]. However, the benefits of this approach remain controversial: although some investigators have reported positive effects on bone formation [17–19], others have failed to detect any improvement [20,21]. This discrepancy in wound-healing outcomes might be explained partly by interindividual variation because PRP is an autologous resource. Moreover, the optimal concentrations of the calcium and thrombin, which are the inducers of platelet activation leading to growth factor release, are unclear [25]. To maximize the healing potential of the application of PRP to bone regeneration, it is necessary to introduce sufficient number of osteogenic stem cells that respond to PRP-derived growth factors by way of autologous bone grafts or the implantation of a cell-scaffold construct [17]. If such a cell source and scaffold can be supplied at the site of the defect, it has been shown by case studies to induce bone formation successfully [17–19]. Further information obtained from basic research at the cellular and molecular level could help this to become a reliable technique for assisting wound healing. The combination of a drug delivery system (DDS) with PRP may be effective, as the following paragraph shows.

The development of synthetic or natural polymer-delivery vehicles for the sustained release of growth and differentiation factors will be crucial for their clinical utility [26,27]. Locally-applied growth factors are defused away rapidly from the implantation site and have a short half-life as a result of a combination of physical and biologic degradation mechanisms. DDS using biomaterial vehicles has allowed the tissue-exposure time to be extended and protein stability to be maintained within the body. Nakahara and colleagues [28,29] investigated the effectiveness of a tissue regeneration device combining a collagen sponge-scaffold material and gelatin microspheres, which prolonged the period of bFGF release in beagles with artificially prepared intrabony periodontal defects. This report was the first to demonstrate the usefulness of DDS and cell scaffolds for targeted periodontal regeneration using a full-scale animal experimental model. In this controlled delivery system, positively charged bFGF molecules formed an ionic complex with acidic gelatin and were released gradually as a result of the degradation of the vehicle in vivo over an extended period. In the bFGF-treated group, 4 weeks after implantation, numerous capillary vessels were observed within the regenerated tissue around the residual gelatin vehicles containing bFGF. This observation indicated that the powerful angiogenic activity of bFGF still was present at this stage. These findings imply that a rich vascular supply is essential throughout the healing process to facilitate periodontal regeneration.

Currently, most delivery approaches involve a single growth factor. These techniques might be unable therefore to induce well-developed vascular networks leading to tissue regeneration, as angiogenesis results from a complicated series of cellular and molecular interactions. However, recent advances in polymeric technologies have allowed the delivery of multiple angiogenic growth factors with distinct kinetics by a single scaffold. For example, Richardson and colleagues [30] reported the development of a new porous polymer scaffold that can deliver vascular endothelial growth factor (VEGF) and PDGF-BB. In this system, VEGF, which stimulates the outgrowth of immature vessels consisting of naked endothelial cells, is mixed with the polymer scaffold, resulting in its rapid release from the vehicle during the first days or weeks after implantation. Meanwhile, PDGF-BB, which stimulates the maturation and stabilization of nascent vessels by way of the recruitment of smooth-muscle cells, is pre-encapsulated within microspheres in the polymer scaffold, from which it is released by degradation in a delayed fashion. The controlled delivery of VEGF and PDGF-BB significantly increases the maturity of the resultant vessel networks. Similarly, Cao and colleagues [31] reported that a combination of PDGF-BB and FGF-2 synergistically induced stable vascular networks, whereas single growth factors were unable to maintain the newly formed blood vessels. Such dual growth factor-delivery technologies could be useful in periodontal tissue engineering.

Cell-based approaches

Cell transplantation is currently a hot topic in the medical field and cell-based therapy using autologous cells is expected to play a central clinical role in the future [32–34]. Several preclinical studies using mesenchymal stem cells (MSCs) have shown efficient reconstruction of bone defects larger than those that would spontaneously heal (that is, critical size defects) [35,36]. Dental cell-seeding studies have attempted to regenerate periodontal tissues since the 1990s [37–41], although clinical applications have become realistic only in recent years.

Kawaguchi and colleagues [42] used bone-marrow-derived MSCs in combination with atelocollagen to regenerate periodontal tissues in experimental class III furcation defects in dogs. After cell expansion for 2 weeks, autologous MSCs mixed with collagen gel were transplanted into the defects. In the MSC-treated groups, significant periodontal tissue structures were observed 1 month after implantation compared with the collagen gel group. However, at the experimental cell concentrations (2×10^6–10^7), no significant difference was observed between the extent of regeneration of the bone and cementum tissues. The investigators concluded that additional studies using different scaffold materials and a various range of cell concentrations would be required to obtain conclusive results. Akizuki and colleagues [43] used autologous periodontal ligament cells obtained from

extracted tooth roots to fabricate cell sheets using a temperature-responsive cell-culture approach based on cell-sheet engineering. A special culture dish, in which the dish surface is hydrophobic under normal culture conditions at 37°C, allowing cells to attach themselves to it and grow but becomes hydrophilic at 20°C so that cells detach themselves spontaneously, was used [44]. This process enabled the collection of the confluent cell cultures as a single sheet in which the deposited extracellular matrix and cell-cell junction proteins remained intact, in contrast to traditional enzymatic treatments for cell detachment, which damage the cultured cells. Autologous periodontal ligament-cell sheets along with a reinforced hyaluronic-acid carrier were implanted into experimental dehiscence defects in dog molars. After 8 weeks, significantly improved periodontal tissue regeneration was observed compared with control cases that received the hyaluronic-acid cell carrier alone. Although further studies are needed to confirm the reproducibility of these regenerative effects, cell-sheet engineering has shown great potential as a new cell-based periodontal therapy.

Cell transplantation has been shown to promote periodontal regeneration compared with the carrier alone as the control. However, it remains unclear whether the transplanted cells differentiate into osteoblasts, cementoblasts, and fibroblasts—to form bone, cementum, and periodontal ligament, respectively—or whether they recruit surrounding host cells to facilitate the regeneration of the periodontal tissues. The fate of the transplanted cells can be determined by labeling them and, thereby, the localization of the transplanted cells within the regenerated tissue can be detected [45].

Cells that are harvested from patients are often frozen and stored for long periods before use in the treatment of periodontitis. Previous studies have demonstrated that cells that have been subjected to freezing and thawing retain the ability to form periodontal tissues, thereby confirming the usefulness of such autologous cells [40–42]. Stem cells are believed to be present in all organs of the body where they maintain tissue homeostasis. The existence of periodontal ligament stem cells (PDLSCs) has been debated for some time. However, one recent study obtained a PDLSC population from the periodontal ligament of human adult impacted third molars [46]. Hence, autologous cells, including PDLSCs, represent valuable patient-derived therapeutic resources for use in the natural reconstruction of tissue destroyed by periodontal diseases.

Gene delivery-based approaches

Numerous tissue regeneration studies have investigated various gene-delivery techniques [47–49]. These techniques involve a gene encoding a therapeutic protein being introduced into cells, which can then express the target protein. This technique avoids the problems associated with the protein-delivery method by maintaining constant protein levels at the site of the defect. Genetic engineering approaches generally consist of two modalities: in vivo

and ex vivo gene delivery. In the former, gene constructs, such as expression plasmid DNA or a viral particle, are physically entrapped within a scaffold or matrix. When the scaffold containing the gene constructs is implanted into the tissue defect, the host cells migrate into the implant, take up the gene constructs and start to produce the encoded protein. By contrast, in the latter approach, cultured cells are transfected (in nonviral delivery systems) or transduced (in viral delivery systems) with gene constructs in vitro before they are transplanted into the tissue defect.

Jin and colleagues [50,51] investigated gene therapy by incorporating BMP-7 and PDGF-B genes into adenovirus vectors. Rat syngeneic dermal fibroblasts were transduced ex vivo with adenoviruses encoding BMP-7 (Ad-BMP-7). These cells were then seeded onto gelatin sponges and placed into periodontal osseous defects [50]. Ad-PDGF-B was used for in vivo direct gene transfer. This vector was initially mixed with a collagen matrix before implantation into rat periodontal alveolar bone defects [51]. These adenoviral gene-transfer approaches stimulated regenerative activities in the periodontal tissues, including osteogenesis, cementogenesis, and periodontal ligament formation. Moreover, in each experiment, minimal periodontal tissue regeneration was induced using Ad-noggin, which is an inhibitor of several BMPs [50], or Ad-PDGF-1308, which inhibits the effects of PDGF activity [51]. Thus, the usefulness of gene therapy for periodontitis has been documented in rats. However, it still remains necessary to confirm the safety and predictability of tissue regeneration that is induced by adenovirus vectors.

Although viral delivery systems have been used successfully in a broad range of tissues, they have some disadvantages, including the risks for mutagenesis, carcinogenesis, and invoking immune reactions in response to viral infection or viral proteins. The development of safer nonviral alternatives is now progressing. Bonadio and colleagues [52] reported that plasmid DNA encoding an active fragment of the human parathyroid hormone physically entrapped within a collagen matrix to form a moldable three-dimensional porous sponge could be used in bone tissue engineering applications. Cells recruited by the repair process took up the plasmid DNA within the collagen matrix and expressed the protein in vivo, which resulted in the generation of bone tissue. Bonadio and colleagues [52] demonstrated the potential utility of this nonviral gene delivery system in critical-size canine skeletal defects.

In a recent study, the sonic hedgehog (Shh) gene, which encodes an essential regulator protein of embryonic osteogenesis and the repair of bone fractures, was transduced into periosteal and fat-derived stem cells, and gingival fibroblasts, to regenerate rabbit cranial bone defects in an alginate and collagen matrix [53]. At 12 weeks postimplantation, a significant increase in bone regeneration was observed in all three Shh-transduced groups compared with the control. This study suggested that the modified cells coordinated the expression of multiple growth factors, such as the BMPs,

implicated in bone formation, and demonstrated the potential use of such a novel gene-enhanced tissue engineering approach in bone regeneration.

In summary, gene delivery-based therapy focused on the regeneration of periodontal and osseous defects has been successful at the experimental level. These findings will encourage the further development of safe and reproducible tissue engineering approaches for clinical application.

Fig. 2 summarizes the tissue engineering approach for the activation of stem cells with the aim of tissue regeneration. Growth factors that are delivered to a local site bind to cell-surface receptors and send intracellular signals. Subsequently, the cells proliferate and differentiate. In addition, gene-transfer techniques might allow the delivery of therapeutic growth factors by way of genetically modified cells, leading to the sustained expression and release of target proteins to the surrounding tissues in vivo, which will further enhance the regenerative potential of stem cells.

Remarks and future directions

Which is more important target for tissue engineering, teeth or the tissues supporting them? In previous decades, numerous studies have investigated the regeneration of periodontal tissues as introduced in the present review. Recently, the focus has shifted, with two studies attempting to regenerate teeth [54,55]. However, although these groups successfully reconstituted the individual structural elements that make up a tooth crown (that is, the dentin, enamel, and dental pulp), neither managed to regulate the morphogenesis of the crown or to regenerate the tooth root. Further improvements

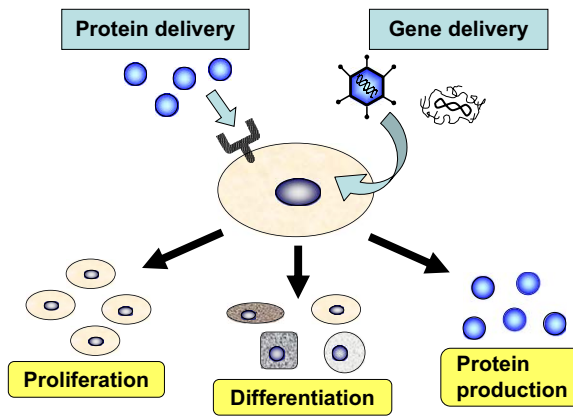

Fig. 2. Schematic representation of activation of stem cells through tissue-engineering approaches. Locally applied therapeutic proteins bind to appropriate receptors displayed at cell surface. Subsequently, cells are activated and undergo proliferation or differentiation. After taking up gene constructs through in vivo or ex vivo gene transfer using a plasmid or viral vector, genetically modified cells can either be seeded or migrate into scaffold, where they continuously secrete transgene-encoded therapeutic proteins into surrounding tissues.

and innovative approaches will be required to reconstruct "complete" teeth using tissue engineering.

Despite the recent interest in tooth regeneration, the view remains that, in a clinical setting, the fundamental goal is actually the regeneration of periodontal tissues, particularly centering on the periodontal ligament. If the periodontal ligament could be regenerated, an artificial tooth implant would suffice, yielding almost natural occlusion and mastication accompanied by real sensation while chewing. It is difficult to judge which theory is correct, but talking to patients about realizing the dream of regenerating teeth has revealed the huge impact that such technology would have on many of their lives. By extension, patient demand clearly defines the ultimate goals for the development of advanced regenerative dental techniques. Thus, the regeneration of teeth is likely to propose an important research topic, which is intimately related to the natural reconstruction of periodontium.

Guided tissue regeneration (GTR) was clinically applied in dental regenerative therapy before any other medical field, and the enamel matrix derivative, Emdogain, is the first periodontal therapy based on a biologic approach. These techniques are examples of first-generation regenerative therapies. The various attempts at tissue regeneration, which come under the general heading of "tissue engineering," introduced in the present review should probably be classed as "second-generation" regenerative medicine. This second-generation approach is making surprisingly rapid progress and its remit has expanded from its original application in the medical field to various related disciplines in which the introduction of biologic and engineering knowledge and skills are allowing the development of new approaches.

In the near future, third-generation periodontal therapies will involve nanoscale science [56–59] and moldless manufacturing technology commonly known as rapid prototyping (RP) [60] or solid free-form fabrication (SFF) [61]. These scientific and technologic innovations will make it possible to fabricate complex scaffolds that mimic the different structures and physiologic functions of natural fibro-osseous tissues, including those, such as periodontium, which consist of hard and soft tissues. The advancement of such technology might also make it possible to produce patient-specific cell-scaffold constructs with optimal distribution of cells and high vascular permeability [62,63].

In the context of regenerative dentistry, the various stem cells existing in human dental tissues are being isolated continuously from adult dental pulp [64], exfoliated deciduous teeth [65], and periodontal ligament [46]. The understanding of the molecular mechanisms that control stem cell function has been improving, although the translational research necessary to apply this knowledge to regenerative medicine is still in its infancy. Future studies will require the collaboration of researchers from different disciplines, including stem cell biology, material science, and of course, basic and clinical dentistry [66–68].

Conventional dental treatment has relied on symptomatic treatment and prosthetic restoration using artificial materials, and clinicians have tended to concentrate on improving their skills rather than developing new modes of treatment. To accelerate the clinical application of newly developed periodontal therapy, the important issues should be addressed while developing the periodontal therapeutic techniques. The ideal system for clinical use will be a simple procedure that provides one-step delivery of the gene/protein of interest with minimal manipulation. The development of such a therapeutic approach will enable wider clinical application that will benefit the increasing numbers of the aging population suffering from periodontitis.

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