Review Article

In Vivo Experiments with Dental Pulp Stem Cells for Pulp-Dentin Complex Regeneration

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In recent years, many studies have examined the pulp-dentin complex regeneration with DPSCs. While it is important to perform research on cells, scaffolds, and growth factors, it is also critical to develop animal models for preclinical trials. The development of a reproducible animal model of transplantation is essential for obtaining precise and accurate data in vivo. The efficacy of pulp regeneration should be assessed qualitatively and quantitatively using animal models. This review article sought to introduce in vivo experiments that have evaluated the potential of dental pulp stem cells for pulp-dentin complex regeneration. According to a review of various researches about DPSCs, the majority of studies have used subcutaneous mouse and dog teeth for animal models. There is no way to know which animal model will reproduce the clinical environment. If an animal model is developed which is easier to use and is useful in more situations than the currently popular models, it will be a substantial aid to studies examining pulp-dentin complex regeneration.

1. Introduction

Since Kakehashi et al. reported that bacteria caused pulpitis in their mouse experiment in 1965, endodontic treatment has developed with the goal of the complete removal of bacteria from the root canal [1]. It was believed that, once exposed to bacteria, the dental pulp would fall into a state of irreversible pulpitis that could never be restored to the normal state and that the progression of inflammation would result in necrosis. Therefore, the dental pulp tissues that are damaged due to bacterial invasion or dental trauma should be removed. The conventional root canal treatment focuses on three-dimensional mechanical preparation, disinfection, and the tight sealing of the root canal space to completely eliminate the dental pulp and bacteria in the root canal and prevent reinfection. However, this treatment is only a measure aimed at repair rather than true healing/regeneration. Thus, many studies have been conducted in the field of dental endodontics with the aim of pulp regeneration.

Two approaches have been undertaken for dental pulp-dentin complex regeneration. The first approach is the revascularization procedure. Many case reports have found clinical success following revascularization procedures, for example, no symptoms and no periapical lesions [2–4]. However, the histological observations from animal experiments have revealed that the tissues formed in the root canal do not reflect the regeneration of pulp-dentin but are rather formed of periodontal tissues, such as cementum, periodontal ligament, and bone [5, 6]. The revascularization procedure has its own clinical advantages in the treatment of immature teeth, but it does not result in pulp-dentin complex regeneration in the true sense.

The second approach for regenerating the dental pulp-dentin complex is the transplantation of mesenchymal stem cells into endodontically treated canals. In recent years, various stem cells have been isolated from the oral cavity. In 2000, Gronthos et al. isolated dental pulp stem cells (DPSCs) from human 3rd molars for the first time, and these
Tissue engineering approaches are based on three central components: the living cells, scaffolds, and growth factors. Various types of stem cells, scaffolds, and growth factors have been researched and reported to function in pulp-dentin complex regeneration. Before applying these candidate substances in clinical trials, their biocompatibilities and treatment efficacies should be evaluated in vivo using animal models. In pulp-dentin complex regeneration research, the absence of animal models that adequately reflect pulp injury and pulpitis for investigation of the use of DPSCs has resulted in skipping preclinical testing. However, if the efficacy and safety of a treatment have not been validated in animal experiments, serious side effects can occur [14, 15]. Thus, the development of a reproducible transplantation animal model is essential for obtaining precise and accurate data in vivo [16]. The efficacy of pulp regeneration should be assessed qualitatively and quantitatively via the use of animal models.

This review article sought to introduce in vivo experiments that have evaluated the possibilities of dental pulp stem cells for pulp-dentin complex regeneration. This paper concentrates on the selection of in vivo and DPSC experiments that were identified in the Medline database via PubMed.

2. In Vivo Engineering in Dental Pulp Regeneration

2.1. Ectopic Transplantation versus Orthotopic Transplantation. The use of preclinical animal models is the most informative approach to obtaining clinically relevant data and verifying safety prior to human application. Animal model designs are categorized into ectopic transplantation and orthotopic transplantation. The word “ectopic” is from the Modern Latin from the Greek word ektopos, which means away from an original place or abnormal position. The word “orthotopic” is from the Greek word orthos, which means normal or usual position.

In pulp-dentin complex regeneration research, the ectopic implantation of stem cell involves transplantation of the cells into the outside of the tooth, for example, into subcutaneous tissue or the renal capsule. Orthotopic implantation involves the transplantation of the cells into the root canal space. Table 1 summarized current in vivo transplantation experiments with dental pulp stem cells.

| Author                  | Animal     | Ectopic/orthotopic | Source of stem cell |
|-------------------------|------------|--------------------|---------------------|
| Shi et al., 2005 [17]   | IC mice    | Ectopic (subcutaneous) | hDPSCs, SHED, hPDLSCs |
| Okamoto et al., 2009 [18]| IC mice    | Ectopic (subcutaneous) | hDPSCs |
| Alongi et al., 2010 [19]| IC mice    | Ectopic (subcutaneous) | hDPSCs |
| Huang et al., 2010 [20]| IC mice    | Ectopic (subcutaneous) | hDPSCs, SCAP |
| Wang et al., 2010 [21]  | IC mice    | Ectopic (subcutaneous) | hDPSCs |
| Galler et al., 2011 [22]| IC mice    | Ectopic (subcutaneous) | hDPSCs, SHED |
| Lee et al., 2011 [23]   | IC mice    | Ectopic (subcutaneous) | hDPSCs |
| Choung et al., 2013 [25]| IC mice    | Ectopic (subcutaneous) | hDPSCs |
| Yu et al., 2006 [26]    | Rats       | Ectopic (renal capsule) | hDPSCs |
| Yu et al., 2007 [27]    | Rats       | Ectopic (renal capsule) | hDPSCs, BMSSCs |
| Yu et al., 2008 [28]    | Rats       | Ectopic (renal capsule) | hDPSCs |
| Iohara et al., 2004 [29]| Dogs       | Orthotopic (canine) | Porcine pulp cells |
| Iohara et al., 2009 [30]| Dogs       | Orthotopic (canine) | SP cells from canine pulp |
| Iohara et al., 2011 [31]| Dogs       | Orthotopic (incisor) | SP cells from canine pulp |
| Iohara et al., 2013 [32]| Dogs       | Orthotopic (incisor) | MDPCs |
| Iohara et al., 2014 [33]| Dogs       | Orthotopic (incisor) | MDPCs |
| Zhu et al., 2012 [34]   | Dogs       | Orthotopic (premolar) | cDPSCs |
| Kodonas et al., 2012 [35]| Mini pigs  | Orthotopic (incisor root, jaw bone) | sDPSCs |

IC, immunocompromised; hDPSCs, human dental pulp stem cells; SHED, stem cells from human exfoliated deciduous teeth; hPDLSCs, human periodontal ligament stem cells; SCAP, stem cells from apical papilla; BMSSCs, bone marrow stromal stem cells; SP, side population; MDPCs, DPSC subfraction based on their migratory response to granulocyte-colony stimulating factor; cDPSCs, canine dental pulp stem cells; sDPSCs, swine dental pulp stem cells.

DPSCs were characterized as highly proliferative cells with self-renewal multidifferentiation properties in vitro [7]. The dental pulp is vascularized and characterized by innervated loose connective tissue that contains heterogeneous cell populations [8]. Due to the complexity of pulpal tissues, no universally accepted protocols or cell types are currently available to assess pulp regeneration. However, a consensus exists regarding the importance of neural and vascular reinnervation for successful pulp regeneration [9]. DPSCs that exhibit pluripotent mesenchymal stem cell characteristics can be easily isolated from teeth [10]. Therefore, the strong angiogenic and neurogenic potentials of DPSCs have attracted much attention in the study of pulp regeneration [11–13].

This review article sought to introduce in vivo experiments with the application of DPSCs for pulp-dentin complex regeneration. This paper concentrates on the selection of in vivo and DPSC experiments that were identified in the Medline database via PubMed.
mediators of inflammation

that odontoblast-like cells proliferate from DPSCs to produce
dentin-like hard tissue on the dentinal walls. This research is
significant in that it used a human root model that created
an environment as similar as possible to that of orthotopic
implantations to overcome the limitations of ectopic implan-
tation. Additionally, research has been performed to assess
the effectiveness of scaffolds and to identify the factors that
influence the differentiation of DPSCs into odontoblasts [21–
23, 25].

In addition to subcutaneous tissues, renal capsules are used
in DPSC research. Yu et al. reported several in vivo stud-
ies using rat renal capsules [26–28]. The renal subcapsular
space is a good site for cell transplantation due to its high graft
uptake rate and abundant blood supply [39]. However, renal
capsules are not used frequently because they are difficult to
access compared with subcutaneous tissues.

2.3. In Vivo DPSCs Experiments: Orthotopic Transplantation.

In dental pulp regeneration research, orthotopic transplan-
tations involve implantation into the teeth, that is, the
original anatomical site. When teeth are used in research,
they can reflect the actual clinical environment better than
ectopic sites such as subcutaneous tissues and renal capsules.
However, it is not easy to implant a mixture of DPSCs and
scaffolds into animal teeth. This procedure requires particu-
larly advanced skills and techniques when using mice or rats,
which have tooth diameters of only 2–5 mm. Therefore, larger
animals, such as dogs and pigs, are often preferred over mice
for this purpose [14].

Many dental experimental studies have been performed
in dogs. Dogs are considered an ideal animal model for
dental research due to the similarities in anatomy, growth
patterns, and pathophysiologies with humans [40]. Dogs are
diphyodonts with deciduous and permanent dentitions. The
composition of the permanent dentition is three incisors, one
canine, four premolars, and two molars in the maxilla and
three molars in the mandible. The beagle is one of the most commonly used
dogs because this breed has a well-adjusted temperament and
is easy to handle due to its proper size [41].

Almost all research into the orthotopic transplantation of
DPSCs has been performed in dogs. Iohara et al. reported
various studies about DPSC implantations in dog teeth [29–
33]. In 2004, the efficacy of BMP2 on the differentiation
of DPSCs into odontoblasts was examined in a canine
amputated pulp model. A surgical exposure was made in
the canine, and the amputation was performed. The upper
incisor pulp was extracted and isolated, and the pulp cells
were enzymatically separated. The pellet of the dog pulp
cells was cultured and applied to the amputated root canal.
Consequently, the transplantation of the BMP2-treated pulp
cells into the amputated root canal stimulated reparative
dentin formation [29]. Subsequently, Iohara et al. studied
several factors that affect pulp regeneration using a surgically
amputated canine pulp model. These authors researched the
potential utility of a subfraction of a side population of
cells, that is, CD31+/CD146+ and CD105+ immunophenotype
cells, from dog dental pulp for angiogenesis/vasculogenesis
and pulp regeneration in a surgically amputated canine
pulp model [30, 31]. These authors demonstrated successful
pulp-dentin complex regeneration in canine teeth. The same group also studied the effects of granulocyte colony-stimulating factor and host age on pulp regeneration [32, 33].

In addition to Iohara, other researchers have conducted research on pulp regeneration in dogs. Zhu et al. investigated the pulp regeneration potential of DPSCs and platelet-rich plasma (PRP) using the surgically amputated canine pulp model established by the Iohara group [34]. Contrary to the expectation that better results would be observed in a DPSC and PRP group than in a blood clot group, histologic examinations did not indicate any differences between the two groups. These findings opposed those from previous in vitro research that predicted that DPSCs plus PRP would play an important role in pulp-dentin complex regeneration because PRP contains many growth factors, and treatment with the appropriate concentration of PRP has been shown to enhance the proliferation and mineralization differentiation of DPSCs [24], which indicates that the results obtained in the laboratory may not lead to results in preclinical animal studies.

Several studies have employed mini pigs. Kodonas et al. demonstrated the regenerative capacity of swine DPSCs seeded in organic and synthetic scaffolds and implanted into the jaw bones of mini pigs [35]. These authors established a root implant model. The root implants were manufactured using the middle part of the roots of freshly extracted swine incisors, and the root canals were filled with scaffolds containing DPSCs and then implanted into the fresh postextraction sockets. This method is not truly orthotopic transplantation. However, it can be used as a valuable animal model for DPSC research in that it attempts to reproduce the clinical environment as much as possible.

2.4. Other Orthotopic Transplantation Models. Although not truly DPSC-based research, other efforts have been made to create an animal model to identify the elements that influence pulp regeneration [42, 43]. Torabinejad performed radiographic and histologic examinations with ferret cuspid teeth over 36–133 days after birth [43]. This author reported that ferret cuspid teeth exhibit anatomical, physiologic, histologic, and pathologic characteristics that are similar to those of human teeth. The ferret has four single-root teeth that are easily accessible for the performance of endodontic procedures and easy to evaluate radiographically and histologically. Torabinejad’s model holds some significance as an animal model because ferrets are easier to handle and are cheaper than dogs and present fewer ethical issues than dogs, which are popular pets.

Other researchers have created orthotopic animal models with small animals instead of large animals, such as dogs, pigs, and ferrets. Simon et al. evaluate mice as an in vivo model for the study of pulpal healing in response to direct pulp capping [42]. These authors proposed the possibilities of orthotopic transplantation research with mouse teeth in preliminary studies despite the difficult accessibility and the small sizes of the teeth. Despite their many advantages as experiment animals, small animals, such as rodents, have been considered to be inappropriate for orthotopic transplantation animal models due to their small teeth [14, 43]. However, it became possible to apply DPSCs to the teeth of small animals and to perform histological and radiological observations of those teeth because of the use of microscopes, the developments in experimental instruments, and the introduction of scaffold-free approaches [42, 44]. Additional research on the development of rodent animal models will aid the development of new orthotopic models.

3. Conclusion

Recently, many studies have approached the pulp-dentin complex regeneration with DPSCs. While it is important to perform research on cells, scaffolds, and growth factors, it is also critical to develop animal models for preclinical trials. Although no human studies have yet been performed, research has been conducted with various types of animals and produced promising results.

According to a review of various studies of DPSCs, the majority of studies have used mouse subcutaneous tissues and dog teeth for animal models. There is no way to know which animal model better reproduces the clinical environment, but the results of an orthotopic transplantation experiment will be more reliable than those of ectopic transplantation experiments. The problem is that orthotopic transplantation research lags behind ectopic transplantation research in both quantity and quality. Sufficient orthotopic transplantation research should be conducted before clinical trials are initiated. If an animal model is developed which is easier to use and more versatile than the currently popular models (e.g., the canine pulp model), that model will be of substantial benefit to research on pulp-dentin complex regeneration.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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