Potential Role of Dentin Sialoprotein by Inducing Dental Pulp Mesenchymal Stem Cell Differentiation and Mineralization for Dental Tissue Repair

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Abstract

Introduction—Dentin sialoprotein (DSP) is a dentin extracellular matrix protein, a unique marker of dentinogenesis and plays a vital role in odontoblast differentiation and dentin mineralization. Recently, studies have shown that DSP induces differentiation and mineralization of periodontal ligament stem cells and dental papilla mesenchymal cells in vitro and rescues dentin deficiency and increases enamel mineralization in animal models.

The hypothesis—DSP as a nature therapeutic agent stimulates dental tissue repair by inducing endogenous dental pulp mesenchymal stem/progenitor cells into odontoblast-like cells to synthesize and secrete dentin extracellular matrix forming new tertiary dentin as well as to regenerate a functional dentin-pulp complex. As DSP is a nature protein, and clinical procedure for DSP therapy is easy and simple, application of DSP may provide a new avenue for dentists with additional option for the treatment of substantially damaged vital teeth.

Evaluation of the hypothesis—Dental caries is the most common dental disease. Deep caries and pulp exposure have been treated by various restorative materials with limited success. One promising approach is dental pulp stem/progenitor-based therapies to regenerate dentin-pulp complex and restore its functions by DSP induction in vivo.

Keywords
Dental caries; Dentin sialoprotein; Cell differentiation; Mineralization; Regeneration
Introduction

Oral and dental diseases are the fourth most expensive disease to treat in developed countries [1]. In 2000 alone, the European Union spent a total of 54 billion European Yuan on oral and dental health care [2]. In USA, expenditure for oral and dental health care in 2004 reached an astounding 81.5 billion dollars [3]. As people are living longer it is becoming increasingly important to sustain and improve their quality of life by maintaining oral and dental functions. Dental caries remains the most prevalent infectious disease in the world. More than 90% of all adults have experienced this disease [4]. Tooth decay can lead to disease of the dental pulp with sequent pulpal infection, necrosis and loss of tooth vitality and possibly function as well as eventually loss of tooth. Various restorative materials have successfully been used to fill and replace diseased or injured dental tissues. However, approximately 50% of cases require revision within 5-10 years after restorative treatment [5]. In addition, any traditional artificial restorative materials might fail due to inappropriate physical, biocompatible and mechanical properties [6-7]. The material pulls away from the cavity wall and microleakage would form between the cavity and dental material. The microleakage may have problems against bacteria invasion, causing recurrent caries [6-7]. Therefore, despite several advances of dental restorative materials, it is required for novel therapeutic restorative approaches in dentistry to maintain a healthy dentition. Therapies using tissue engineering, stem cells and growth factors have successfully been reported by regenerating or replacing diseased and injured dental tissues [6-13]. Thus, it will provide a potential avenue for clinical application of such therapies. Our hypothesis is that use of dentin sialoprotein (DSP) protein as a nature, novel agent to induce endogenous dental pulp stem/progenitor cell differentiation into odontoblast-like cells and to facilitate regeneration and repair of dental diseases.

Dentine formation and repair

Dentin consists of dentinal tubules and intertubular dentins. Dentinogenesis (dentin formation) is responsibility of odontoblasts that are long-living mitotic cells situated around the periphery of the dental pulp chamber. The odontoblasts differentiate from ectoemsenchymal cells as well as synthesize and secrete dentin extracellular matrix (DECM) that become mineralized to form dentin. The dental pulp is the soft connective tissue that supports dentin and is considered the vital hub of the tooth. It not only functions to provide nutritional and sensory properties to dentin, but also has its own reparative capacity. The principal cells of the dental pulp are the odontoblasts, capillary endothelial cells, fibroblasts and mesenchymal stem/progenitor cells forming an intricate system with the dentin-pulp complex that functions as a homeostatic tissue capable of physiological repair. This feature plays an important role in dentin repair and regeneration whereby odontoblasts respond to tooth injury and produce DECM forming tertiary dentin. The endogenous process of dental tissue repair is essential to restore lesions of the dentin forming a biophysical barrier effectively sealing off lesions and providing protection for the vital pulp tissue [7,14-15]. Advanced dental caries and invasive restorative procedures are major tooth damages that can irrevocably injury and even cause odontoblast cell death, thereby obliterating odontoblast-mediated tissue repair. However, the dentin-pulp system holds an endogenous repair mechanism that can be activated following tooth decay or trauma [7,16]. This repair process is involved in activating and inducing differentiation of mesenchymal stem/progenitor cells into odontoblast-like cells that produce DECM, particularly during reparative dentinogenesis associated with tooth injury and diseases [7,8,16,17].
**Dentin sialophosphoprotein (DSPP)**

The DECM is composed of the inorganic components and organic matrix which consists of collagenous and non-collagenous proteins (NCPs). Among the NCPs, dentin sialophosphoprotein (DSPP) is the most abundant non-collagenous protein in dentin and acts as a unique marker of odontoblast differentiation and dentin mineralization [18,19]. This demonstrates that a functional role of DSPP is mainly involved in tooth formation and mineralization. Mutations of the DSPP gene cause various types of dentinogenesis imperfecta [20-25] and are the most common hereditary dentin disorders in human being [26-28]. Patients with these diseases present with discolored teeth, enlarged pulp chambers that fill in with mineralized matrix, a wider pre-dentin zone, decreased dentin width, hypomineralization and the prevalence of pulp exposures [20-23]. DSPP is a precursor that is proteolytically processed into two major dentin matrix proteins; dentin sialoprotein (DSP) and dentin phosphoprotein (DPP) [29-32]. Either DSP or DPP plays distinct biological functions during tooth development and formation [33,34]. In vivo studies have shown that DSP regulates initial dentin mineralization and contributes to the mechanical event of dentin-enamel junction (DEJ) [33,34]. Furthermore, studies have shown that recombinant DSP protein is capable of inducing human dental periodontal ligamental (PDL) stem cell and mouse dental papilla mesenchymal cell differentiation and mineralization [35]. Mechanisms of DSP effect on cell differentiation and mineralization may be involved in p38 and Smad 1/5/8 signaling transduction pathways via interactions with cell membrane receptors, CD105 and integrin beta 6 [35]. Based on our and other observations, we postulate the DSP may be used as an effective stimulator for dental pulp stem/progenitor cell differentiation and dental tissue repair.

**The hypothesis**

Microenvironments (niches) influence cell behavior and fate [36,37]. For instance, bone ECM influences osteoblast differentiation into osteocytes while dental ECM governs dental pulp mesenchymal stem cell differentiation into odontoblast cells [38-42]. As the most abundant DECM non-collagenous protein, DSP displays its biological functions through the cellular membrane receptors, inducing dental pulp stem/progenitor cell differentiation to form dentin. In human, 90% of DSPP gene mutations occurred in DSP domain, causing various types of dentin genetic diseases [23,26]. Our hypothesis is that DSP as a nature peptide may be used as a dental therapy to induce dental pulp stem/progenitor cell differentiation and to promote tooth tissue repair and dental pulp regeneration. We postulate that adding recombinant DSP protein to dental deficient site will be able to stimulate endogenous tissue repair responses within dental pulp, inducing differentiation of dental pulp mesenchymal stem/progenitor cells into odontoblast-like cells, thereby forming tertiary dentin.

Our previous work showed that recombinant DSP induces differentiation and mineralization of dental PDL stem cells and dental papilla mesenchymal cells [35]. Suzuki et al. found that DSP regulates initial dentin mineralization and partially rescue the dentin volume with the restored pre-dentin as well as decreases frequent dental pulp exposure in DSPP homogenous null mice [34]. Furthermore, studies by Paine et al. demonstrated that DSP accelerates rate of enamel formation, contributing to the mechanical event of dentin-enamel junction [33].

Our hypothesis is that DSP is an effective and nature therapeutic agent without any side effects to stimulate dental pulp regeneration, dentin formation and tooth tissue repair. In this context, it is to postulate that during restorative procedure DSP is implanted into dental carious or injury area (s) and induces dental pulp mesenchymal stem/progenitor cell differentiation and mineralization and contributes to the mechanical event of dentin-enamel junction. (DEJ) [33,34]. Furthermore, studies have shown that recombinant DSP protein is capable of inducing human dental periodontal ligamental (PDL) stem cell and mouse dental papilla mesenchymal cell differentiation and mineralization [35].
differentiation into odontoblast-like cells, which produce and secrete DECM to form new tertiary dentin, therefore “sealing off” the dental carious or injury area(s).

On the other hand, this strategy is also tempting advantage that the DSP-induced odontoblast cells are capable of migrating to the microleakage region between the cavity wall and restorative dental material, forming new reparative dentin, sealing off the gap and preventing bacteria invasion from recurrent caries.

**Evaluation of the hypothesis**

We have demonstrated that in vitro recombinant DSP induces differentiation and mineralization of PDL stem cells and dental papilla mesenchymal cells when DSP protein was added within medium containing these cells [35]. It is quite promising to investigate the impact of DSP to stimulate tooth repair and regeneration as part of restorative dentistry for clinical benefit in future. There is a strategy for use of DSP protein to regenerate dentin: DSP gene is subcloned into the glutathione S-transferase (GST) expression vector (GE Healthcare Biosciences, Piscataway, NJ, USA). Purification of GST-DSP fusion protein is performed by Glutathion Sepharose 4B system (GE Healthcare Biosciences). PreScission protease cleaves the junction between GST and DSP and releases DSP protein, but the protease and GST protein remain in the beads. The purified recombinant DSP is incubated with Affi-Gel blue affinity gel that is a beaded, crosslinked agarose gel with covalently attached Cibacron® Blue dye (Bio-Rad, Hercules, CA, USA), and DSP protein is absorbed by the microbeads, forming the DSP-soaked agarose beads. After cleaning the carious area, the DSP-soaked agarose beads are implanted into the carious area, and then the cavity is sealed off with light-cured glass-ionomer cements (Fuji II, GC Corporation, Tokyo, Japan). DSP protein is gradually released from the bio-degradable synthetic gel and induces the endogenous dental pulp stem/progenitor cell differentiation into odontoblast-like cells and then these cells synthesize and secrete dentin ECM, thereby regenerating a functional pulp-dentin complex and accelerating tertiary dentin formation in vivo.

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**List of abbreviations**

- **DSP**: Dentin sialoprotein
- **DECM**: Dentin extracellular matrix
- **NCPs**: Non-collagenous proteins

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