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Dentine-pulp tissue engineering in miniature swine teeth by set calcium silicate containing bioactive molecules

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ABSTRACT

Objective: : The present study aims to investigate whether reparative dentinogenesis could be guided at central pulpal sites or at a distance from the amputated pulp of miniature pig teeth, by using set calcium silicate-based carriers containing human recombinant bioactive molecules.
Design: Pulp exposures were performed in 72 permanent teeth of 4 healthy miniature swine. The teeth were capped with pre-manufactured implants of set calcium silicate-based material containing BMP-7, TGFβ1 or WnT-1, for 3 weeks. Conical-shaped intrapulpal implants were exposed in the central pulp core, while disc-shaped extrapulpal implants were placed at a distance from the amputated pulp. Implants without bioactive molecules were used as controls. Thickness and forms of new matrix mineralized deposition were assessed histologically at post-operative periods of 3 weeks by light microscopy.
Results: Intrapulpal applications: Calcified structures composed of osteodentine were found in contact with the BMP-7 implants. An inhomogeneous calcified tissue matrix was found around the WnT-1 carriers. A two-zone calcified structure composed of osteodentine and a thicker tubular matrix zone was seen at the TGFβ1 carrier-pulp interface. Extrapulpal applications: The space between WnT-1 implants and pulp periphery had been invaded by soft tissue with traces of calcified foci. Thick calcified structures composed of osteodentine were found surrounding pulp exposure sites in response to application of BMP-7. Spindle-shaped cells associated with atubular calcified matrix or elongated polarized cells associated with tubular dentine-like matrix were found along the cut dentinal walls of the TGFβ1 group.
Conclusion: The present experiments indicated that set calcium silicate could be used as carrier for biologically active molecules. TGFβ1 was shown to be an effective bioactive molecule in guiding tertiary dentine formation.

1. Introduction

It is widely recognized that traumatized dental pulp possesses the ability to form a dentine-like matrix (reparative dentine) by elongated and polarized cells (odontoblast-like cells) differentiating in the absence of normal developmental conditions, i.e., enamel epithelium and/or basement membrane (Baume, 1980; Rutherford, 1999). In biological terms, reparative dentinogenesis can take place in different ways: as a part of the wound healing process in an inflammation-free pulp environment without any therapeutic intervention (Kakehashi, Stanley, & Fitzgerald, 1965), or in the presence of a wide spectrum of dental materials (Cox, 1987), or in response to various signaling molecules (Rutherford, 1999; Tziafas, Smith, & Lesot, 2000).

Therapeutic challenges to exploit the reparative dentinogenic potential of the dentine-pulp complex and thus maintain pulp vitality and function are included in the so-called vital pulp therapy. The ultimate goal and therefore the most important determinant for the maintenance of pulp vitality following vital pulp therapy is the restoration of normal tissue architecture at the amputated dentine-pulp interface. Since primary dentine cannot be formed post-developmentally, reparative dentine is the most appropriate extracellular matrix capable of effectively opposing exogenous destructive stimuli at the pulp periphery. Reconstitution of the injured pulp-dentine interface with directed induction

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of reparative dentine formation at the therapeutic application-pulp interface has been repeatedly demonstrated. This represents the end-result of a pulp repair function that can provide a necessary barrier effect. However, it is an unpredictable tissue response which usually occurs at the expense of the limited pulp space (Murray, Smith, Garcia-Godoy, & Lumley, 2008; Smith, 2003). The crucial role of exogenous stimuli in the pulpal wound healing process and subsequent hard-tissue formation is well recognized (Baume, 1980; Schroder, 1985). In traditional pulp therapies, a low-grade chemical irritation resulting from the therapeutic application of a capping material enhances the natural healing capacity of the underlying pulp. As a part of the natural healing process, extracellular matrix is deposited; initially appearing as atubular or osteotypic hard tissue and progressively becoming lined with reparative dentine (Cvek, Granath, Cleaton-Jones, & Austin, 1987; Schroder, 1985). The involvement of growth factors and other pulp or dentinal tissue extracellular matrix molecules, in the signaling and regulation of dentinogenic events has provided a master plan for development of new therapeutic applications in vital pulp therapy.

In numerous experimental studies, the application of signaling molecule(s)-based delivery systems have demonstrated induction of atubular hard tissue formation and reparative dentine, beyond the stereotypical defensive mechanism. Experimental data indicate that both factors, the signaling molecules and the presence of an appropriate mechanical support or substratum, seem to be of critical importance for the desired end-result of the repair process in the pulp-dentine complex (Rutherford, 1999; Smith et al., 1995; Tziafas et al., 2000). According to the generally accepted working hypothesis, competent pulpal progenitor cells (a term used for pulpal cells of unknown identity and location, but responsive to appropriate exogenous influences) can be directed along different pathways, depending on the pulp environment, nature and specificity of the exogenous stimuli and/or the existence of an appropriate mechanical support. In an inflammation free pulp environment and appropriate stimuli-substratum conditions, competent pulpal cells migrate and attach to the exposed mechanical support; biomechanical and/or molecular signals to the immobilized cells may stimulate cytological changes and synthesis of extracellular matrix components in a polar predentine-like pattern (Lesot et al., 1994; Tziafas et al., 2000). Among the molecular signals involved in initiation of odentinogenic events in both developmental or post-developmental conditions, Bone Morphogenetic Protein-7 (BMP-7), or Transforming Growth Factor (TGF-β1), or Wnt Signaling Protein (Wnt)-1 have been documented as stimulating proliferation, migration, attachment, differentiation of dental papilla or dental pulp cells and predentin matrix synthesis (Rutherford 1999; Smith 2003; Tziafas et al., 2000).

The present study aimed to investigate pulp responses to a set calcium silicate-based substratum containing bioactive molecules with well known bioactivity, at central pulpal sites or at a distance from the amputated pulp. The experimental model of mechanically exposed pulps of healthy miniature swine teeth, and the human recombinant molecules of BMP-7, or TGF-β1, or Wnt-1 were used.

2. Materials and methods

2.1. Ethical approval

This study was approved by the Ethical Committee of the School of Dentistry, Aristotle University of Thessaloniki, Greece. The experimental study was carried out in accordance with the Ethical Guidelines of the Aristotle University of Thessaloniki (European Communities Directive of 24 November 1986 – 86/609/EEC), for the care of animals in experimental procedures and approved by the Ethical Committee of the School of Dentistry, Aristotle University of Thessaloniki, Greece.

2.2. Animals and experimental procedures

Four healthy miniature swine, all 18–24 months of age, with intact dentitions were used for the present experimental work. All measures were taken to minimize pain or discomfort of the animals.

Each animal was sedated with an intramuscular injection of 1 mg/kg xylazine. General anaesthesia was induced with an intramuscular injection of 6 mg/kg theopentone. Before the beginning of all experimental procedures the trachea was intubated and general anaesthesia was maintained using halothane (1.5%–2.5%) in oxygen, delivered through a semi-closed breathing circuit.

Seventy-two teeth (24 premolars, 24 molars, 16 canines and 8 third incisors) of both jaws were selected for experimentation. Preoperative radiographic examination showed fully developed roots in all teeth. The teeth were scaled and polished with a rubber cup on the day of the operative procedures, isolated with cotton rolls and cleaned with an iodine solution (5%), while saliva was controlled with high-speed evacuation.

Class V cavities were prepared on the buccal surface of incisors and canines, and class I cavities on the occlusal surface of premolars and molars using a tungsten carbide pear-shaped bur, (ISO #330 L SS. White, Lakewood NJ U.S.A.) at ultra-high speed with copious water spray. The active tip of the bur was limited to 1.4 mm and the dimensions of the cavities were approximately 2.0 mm wide, 2.0 mm long and 2.0 mm deep. In the cavities, pulpal exposures were further performed at the middle of the cavity floor using a round carbide bur 0.8 mm in diameter (ISO #1; ShofuInc, Kyoto, Japan) at high speed and under water cooling. A new bur was used for each tooth. The pulp exposures produced were of size 1.5 mm for 36 teeth, or 0.8 mm for the others. The cavities were washed with sterile saline; the haemorrhage was controlled by using wet cotton pellets with light pressure, dried with dry cotton pellets, and capped immediately by using set calcium silicate carriers of biologically active molecules as experimental capping materials.

2.3. Bioactive materials

Carriers: The carriers were manufactured by using a calcium silicate-based material (Septodont, Saint-Maurdes-fosses Cedex, France) set at 4 °C in air environment for one day. The surface structure of set calcium silicate material was analyzed using Scanning Electron Microscopy (JEOL JSM-840) and X-ray spectroscopy. In Fig. 1 a grainy structure of the set material showing peaks in calcium, silicon and chlorine was observed. No crystal precipitation was seen. The form of the carriers and the mode of application were performed in two different ways:

1. Intrapulpal application: Cone–shaped calcium silicate vehicles placed via the 1.5 mm exposures in contact with the central pulp tissue (Fig. 1a) in 36 teeth. The dimensions of the cones were 1.5 mm in diameter and 2 mm in length. The teeth were randomly assigned to 4 groups of 9 teeth each, including 4 molars, 3 premolars and 2 canines and capped with the carriers containing PBS (I-1 group), PBS + BMP-7 (I-2 group), PBS + TBFβ1 (1-3 group) and PBS + WnT-1 (I-4 group).
2. Extrapulpal application: Disc-shaped calcium silicate vehicles placed in the base of the dentinal cavities at a distance from the 0.8mm pulp exposures (Fig. 1b) in 36 teeth. The teeth were randomly assigned to 4 groups of 9 teeth each, including 2