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Zirconia-incorporated zinc oxide eugenol has improved mechanical properties and cytocompatibility with human dental pulp stem cells

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ABSTRACT

Objective. Zinc oxide eugenol (ZOE) is widely used as a therapeutic dental restorative material. However, ZOE has poor mechanical properties and high cytotoxicity toward human dental pulp stem cells (hDPSCs) due to the release of Zn ions. In this study, zirconia-incorporated ZOE (ZZrOE) was developed to reduce the cytotoxicity and improve the mechanical properties of ZOE with sustained therapeutic effects on inflamed hDPSCs in terms of inflammatory gene expression levels compared with those of the original material.

Methods. After the setting time and mechanical properties of ZZrOE incorporating varying amounts of zirconia (0, 5, 10, and 20 wt% in powder) were characterized, the surface morphology and composition of the resulting ZZrOE materials were investigated. The ions and chemicals released into the cell culture medium from ZOE and ZZrOE (3 cm^2/mL) were measured by inductively coupled plasma atomic emission spectroscopy and gas chromatography, respectively. After testing cytotoxicity against hDPSCs using the above extracts, the therapeutic effects on lipopolysaccharide-inflamed hDPSCs in terms of compromising the upregulation of inflammatory response-related mRNA expression were tested using real-time PCR.

Results. ZZrOE 20% exhibited increased compressive strength (~45%), 3-point flexural strength (~150%) and hardness (~75%), as well as a similar setting time (~90%), compared with those of ZOE. After the rough surface of ZZrOE was observed, significantly fewer released Zn ions and eugenol (~40% of that from ZOE) were detected in ZZrOE 20%. ZZrOE showed less cytotoxicity because of the lower amount of Zn ions released from ZOE while showing sustained inhibition of inflammatory marker (e.g., interleukin 1β, 6 and 8) mRNA levels.

Significance. The improved mechanical properties and cytocompatibility, as well as the sustained therapeutic effects on inflamed hDPSCs, were investigated in ZZrOE compared with

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those of ZOE. Therefore, ZZrOE has the potential to be used as an alternative to ZOE as a dental restorative material.

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1. Introduction

Zinc oxide eugenol (ZOE) cement has been used in clinical dentistry for over 100 years as a base material in temporary restoration and cementation due to its low cost, ease of handling, and reasonable sealing, insulating and antibacterial properties [1,2]. In addition, ZOE-incorporated endodontic sealers and temporary ZOE packing have been used in clinical settings to heal dry sockets [3,4]. ZOE is set by mixing zinc oxide (ZnO) powder with liquid eugenol in an acid–base reaction that results in the formation of a zinc eugenolate chelate matrix in which unreacted ZnO acts as a reinforcement [5]. However, this material exhibits poor mechanical properties, which can cause failure of dental/medical restoration in patients with a strong bite force in general dentition or a major point of occlusion on the applied tooth [6,7]. In addition, the cytotoxicity of ZOE against cells that originate from pulp tissue somewhat limit its application as a base material, especially when the ZOE is positioned in deep cavities near the pulp tissue. ZOE extract in dentinal fluids might adversely affect pulp (stem) cells via open dentinal tubules during or after setting [8].

One approach used to overcome these issues is the incorporation of additives into either ZOE powder or liquid. For example, polymer (resin)-reinforced ZOE (i.e., IRM) and ethoxybenzoic acid-alumina-reinforced ZOE show improved mechanical properties and have been used in clinical settings, with beneficial outcomes [9,10]. However, caution should still be taken when applying these materials to deeply prepared cavities [11]. Due to high amounts of released Zn ions during or after setting, these materials might adversely affect the viability of pulp (stem) cells via open dentinal tubules, thereby resulting in the diminishing potency of dentin-pulp complex regeneration [8,12,13]. Therefore, improving the mechanical properties, as well as the cytocompatibility with pulp stem cells, the major cell type of pulp tissue that regenerates the dentin-pulp complex, through the release of fewer Zn ions is necessary to overcome the limited application of ZOE-based dental materials.

Among many types of additives, micro-sized zirconia (ZrO_2) was chosen for incorporation into ZnO powder due to its intrinsic mechanical properties, excellent cytocompatibility, and because this additive is non-allergenic, inert, and non-corrosive. Moreover, ZrO_2 might release fewer cytotoxic zinc ions when incorporated into ZOE [14,15]. Few previous studies have incorporated additives into ZOE except for resin and alumina, as mentioned above; however, these materials only showed improved mechanical properties compared with those of ZOE, and no comparison was performed in terms of biological properties, such as cytocompatibility and therapeutic effects against inflamed pulp stem cells [16]. Therefore, this study is the first to investigate the mechanical properties and biological effects of modified ZOE compared with those of ZOE. Thus, these findings will provide insights for the development of improved ZOE-based dental materials.

The aim of this study was to develop zirconia-incorporated ZOE-based products with improved mechanical properties and cytocompatibility with human dental pulp stem cells (hDPSCs) that maintain their therapeutic effects against inflamed pulp stem cells of ZOE. After the physico-mechanical properties of commercially available ZOE cements (ZOE and IRM) containing different amounts (up to 20 wt%) of incorporated zirconia (ZrO_2) were compared with those of ZOE, the biological effects of these materials were also evaluated. Our first null hypothesis states that the physico-mechanical properties and cytocompatibility of zirconia-incorporated ZOE (ZZrOE) and IRM do not differ significantly from those of ZOE and IRM without zirconia. Our second null hypothesis states that ZZrOE and ZOE would show different therapeutic effects against inflamed hDPSCs in terms of compromising the upregulation of inflammatory-related mRNA expression.

2. Materials and methods

2.1. Synthesis of ZZrOE

Commercially available ZOE, consisting of ZnO powder and 100% liquid eugenol was obtained (Kemdent, Purton, UK; lot numbers 23705 for powder and 22417 for liquid). ZrO_2 (5 μm size, Sigma, St. Louis, MO, USA, lot number: 230693) was incorporated as an additive in quantities of 5%, 10% or 20% by weight relative to the amount of powder. After mixing the materials mechanically using a rolling ball mill for 24 h, liquid eugenol was added to the powder at a ratio of 1:5 liquid (mL) to powder (g). The mixing protocol was conducted in accordance with the manufacturer's instructions. A summary of the experimental groups is presented in Table 1. ZrO_2 was also incorporated into IRM (Dentsply, York, PA, USA) using powder (lot number: 1407283) and liquid (lot number: 1407212) in the same manner as described above for ZZrOE. All materials were properly stored according to the manufacturer's recommended conditions before the experiments. Unless otherwise specified, the specimens were polished using up to 1200 grit SiC paper before use in all experiments.

2.2. Setting time measurement

The setting time was measured according to the ISO standards for ZOE cements (ISO 3107) [17]. Briefly, 60 s after the cement mixing was started, a metal mold (Ø = 10 mm, d = 2 mm) was filled and placed on a metal block, and the cement was conditioned at 37 °C and 95% humidity during the measurements. A flat-end indenter needle (400 g, Ø = 1 mm) was positioned on the cement surface at 15 s intervals starting 8 min after the

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