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Nanocrystalline calcium sulfate/hydroxyapatite biphasic compound as a TGF-β1/VEGF reservoir for vital pulp therapy

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ABSTRACT

Objectives. Vital pulp therapy aims to treat reversible pulpal injuries *via* protective dentinogenesis and to preserve more tooth structure. Mineral trioxide aggregate (MTA)-based capping materials demonstrate prolonged setting time increases the risk of pulpal infection during multi-visit treatment. Their non-degradable property occupies pulp space and limits dentin-pulp regeneration. This study reports an inorganic degradable biomaterial that presents a short initial setting time and acts as a growth factor reservoir to promote reparative dentinogenesis.

Methods. We synthesize nanocrystalline calcium sulfate hemihydrate (nCS), hydroxyapatite (HAp) and calcium sulfate hemihydrate (CS) as a reservoir to which transforming growth factor-beta 1 (TGF-β1) and vascular endothelial growth factor (VEGF) are added (denoted as nCS/HAp/CS/TGF-β1/VEGF). *In vitro* biocompatibility and mineralization (the activity and expression of alkaline phosphatase, ALP) were evaluated. Rat animal model was created to test *in vivo* efficacy.

Results. Cultured human dental pulp cells (HDPCs) showed that nCS/HAp/CS/TGF-β1/VEGF cement has excellent biocompatibility and the potential to elevate the activity and expression of ALP. The *in vivo* efficacy (rat animal model) indicates protective dentin by micro-computed tomography (μ-CT) measurements and histological analyses. The 3D μ-CT non-destructive analysis also determines volume changes during pulpotomy, suggesting that the degraded space of the nCS/HAp/CS/TGF-β1/VEGF cement is repaired by the formation of dentin-pulp tissue.

Abbreviations: nCS, nanocrystalline calcium sulfate; CS, calcium sulfate; HAp, hydroxyapatite; TGF-β1, transforming growth factor-beta 1; VEGF, vascular endothelial growth factor; HDPCs, human dental pulp cells.

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Significance. These findings demonstrate that nCS/HAp/CS cement acts as a potent reservoir for the sustained release of growth factors, and that nCS/HAp/CS/TGF-β1/VEGF cement has a high potential to form the reparative dentinogenesis *in vivo*.

1. Introduction

Teeth with deep caries, traumatic injuries or other damage may lead to dental pulp inflammation or exposure. The traditional treatment for tooth pulp injury is root canal therapy. Although the success rate of root canal therapy is nearly 90% in a 2- to 4-year follow-up study [1], the access opening and shaping of root canal will lose certain amount of hard tissue structure of tooth during the treatment or post space fabrication which may increase the risk of tooth fracture [2–4]. Thus, preservation of remaining pulp vitality *via* direct/indirect/pulpotomy vital pulp therapy can be regarded as minimally invasive dentistry [5,6]. Direct pulp capping therapy is an operation on the floor of cavities after the removal of deep carious lesions or after traumatic exposure, whereas pulpotomy therapy operates on the radicular pulp after removal of the coronal tissue. Three factors are crucial for successful vital pulp therapy: (1) remove harmful challenges to control the infection; (2) adopt a capping biomaterial to protect and stimulate the pulp dentinogenic response; and (3) provide a good seal to prevent bacterial microleakage [7]. Direct vital pulp therapy uses dressing or capping biomaterials to form a protective layer over the exposed vital pulp tissue in pulp capping procedures or pulpotomies. These protective biomaterials should possess specific biological ability and biocompatibility, to promote dental pulp cell activity and form the reparative dentin [8].

The dental pulp contains proliferative stem/progenitor cells, which possess self-renewal and differentiation abilities. These cells may differentiate into odontoblast-like cells and secrete dentin matrix when dental pulp cells are affected by extracellular matrix molecules or external stimulation [9]. It is well recognized that throughout the process, growth factors in the extracellular matrix are critical for inducing odontoblast differentiation in dentinogenesis [10]. Therefore, transforming growth factor-beta 1 (TGF-β1) and angiogenic growth factors, such as vascular endothelial growth factor (VEGF), have been used as potential inducing factors for dentin-pulp complex engineering [11,12]. The major effect of TGF-β1 on odontoblasts was to trigger differentiation and improve dentin or dentin-like hard tissue generation during the healing process. The key role of VEGF was to induce human dental pulp cells to develop into endothelial cells and undergo osteogenetic differentiation [13]. However, the short half-life of growth factors prevents the expected biological effect from being realized, as they can easily be degraded by enzymes [14]. Thus, research aimed at prolonging the half-life of growth factor is being widely pursued [15,16]. Studies have proven that biodegradable polymers acting as carriers can effectively control the release of growth factors [17]. Yuan et al. found that biocompatibility, toxicity, and degradation are crucial considerations in choosing polymers for the capping material [18]. Approaches to vital pulp therapy using organic (*e.g.*, silk fibroin, dentin matrix) or inorganic cements as pulp capping agents have been applied for different purposes [19–21]. Organic materials were less irritating and exhibited good biocompatibility, but most of them were provided as scaffolds with stem cells added to vitalize pulp cells. Inorganic materials may either provide strong mechanical properties and good bacterial sealing or cause pulp inflammation and reduce dentin-pulp complex formation. For example, conventional calcium hydroxide-based materials have been clinically used as pulp capping materials for reparative dentinogenesis for approximately 50 years. However, high solubility and fast dissolution are typical drawbacks that make them clinically inadequate for covering bleeding points and hinder their long-term viability [22]. Mineral trioxide aggregate (MTA) cements, a well-known type of calcium silicate-based cements in dentistry, are hydraulic self-setting materials mainly composed of dicalcium and tricalcium silicates. Their peculiar intrinsic properties, forming calcium hydroxide during their hydration process and setting in the presence of blood and other biological fluids, ensuring a good seal, make them suitable for clinical use [23]. However, their unfavorable healing properties, prolonged setting time, and insufficient ability to promote dentin-pulp regeneration limit their application [24].

In this study, we synthesized an inorganic biomaterial containing nanocrystalline calcium sulfate (nCS), 20–50 μm particles of hydroxyapatite (HAp) and calcium sulfate (CS) – denoted as nCS/HAp/CS – to carry growth factors (TGF-β1 and VEGF) and to provide a substantial release reservoir. HAp, the major inorganic component of enamel and dentin, is well known for its high biocompatibility and improves the material's mechanical strength [25]. CS is a highly biocompatible material and is used as a drug release material for antibiotics, growth factors and pharmacological agents [26]. In addition, to increase the growth factor adsorption, we nanosized calcium sulfate to form nanoparticles with diameters of 80–100 nm. The purpose of our study is to take advantage of each material to maintain pulp vitality, seal the infected channel to avoid irritation from microorganisms and regenerate the dentin-pulp complex. We investigated the efficacy of this synthesized biomaterial by examining its biocompatibility and bioactivity *in vitro* as well as its new reparative dentinogenesis *in vivo*.

2. Materials and methods

2.1. *Preparation of nCS/HAp/CS/TGF-β1/VEGF biomaterials*

The synthesis of nanocrystalline calcium sulfate (nCS) was based on the cryo-vacuum method. A dilute solution of