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Development of radiopaque, biocompatible, antimicrobial, micro-particle fillers for micro-CT imaging of simulated periodontal pockets

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ABSTRACT

Objectives. Approximately 10^9 bacteria can be harbored within periodontal pockets (PP) along with inflammatory byproducts implicated in the pathophysiology of systemic diseases linked to periodontitis (PD). Calculation of this inflammatory burden has involved estimation of total pocket surface area using analog data from conventional periodontal probing which is unable to determine the three-dimensional (3-D) nature of PP. The goals of this study are to determine the radiopacity, biocompatibility, and antimicrobial activity of transient micro-particle fillers *in vitro* and demonstrate their capability for 3-D imaging of artificial PP (U.S. Patent publication number: 9814791 B2).

Methods. Relative radiopacity values of various metal oxide fillers were obtained from conventional radiography and micro-computed tomography (μCT) using *in vitro* models. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were used to measure the biocompatibility of calcium tungstate ($CaWO_4$) particles by determination of viable keratinocytes percentage (%) after exposure. After introducing an antibacterial compound (K21) to the radiopaque agent, antimicrobial tests were conducted using *Porphyromonas gingivalis* (*P. gingivalis*) and *Streptococcus gordonii* (*S. gordonii*) strains and blood agar plates.

Results. $CaWO_4$ micro-particle-bearing fillers exhibited an X-ray radiopacity distinct from tooth structures that enabled 3-D visualization of an artificial periodontal pocket created around a human tooth. MTT assays indicated that $CaWO_4$ micro-particles are highly biocompatible (increasing the viability of exposed keratinocytes). Radiopaque micro-particle fillers combined with K21 showed significant antimicrobial activity for *P. gingivalis* and *S. gordonii*.

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Significance. The plausibility of visualizing PP with 3-D radiographic imaging using new radiopaque, biocompatible, transient fillers was demonstrated *in vitro*. Antibacterial (or other) agents added to this formula could provide beneficial therapeutic features along with the diagnostic utility.

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1. Introduction

Periodontitis (PD) is an inflammatory disease of bacterial origin that can culminate in tooth loss [1,2]. PD is also associated with multiple systemic diseases, such as cardiovascular diseases, diabetes mellitus, cancer, and Alzheimer's diseases [3]. Indeed, nearly 50% of the U.S. population is affected by PD [4].

The loss of the epithelial/connective tissue attachment of the gingiva to the root surfaces, the apical migration of junctional epithelium, and the subsequent formation of the periodontal pocket (PP) are histopathological features of PD. The PPs are sites of infection with predominantly anaerobic oral pathogens, leading to inflammation that represents a considerable infectious/inflammatory burden on the host [5]. However, an accurate estimation of such burden is frequently overlooked [6]. Recent efforts to assess the inflammatory burden posed by PD have involved a calculation to estimate the total surface area of periodontal pockets (periodontal inflamed surface area, or PISA) [7]. However, this technique relies on analog data generated by periodontal probing which does not provide a precise three-dimensional (3-D) assessment of the PP shape and volume [8–13]. The accurate diagnosis, prognosis, and treatment planning, as well as long-term maintenance of PD patients could benefit from 3-D digital imaging technology, which could provide more rapid and precise measurements of both the depth and the 3-D nature of the PP. To the best of our knowledge, however, there is no commercially-available technology to image and measure the 3-D shape and volume of periodontal pockets.

Conventional PP evaluation has been conducted for over 70 years by manual probing of ~192 sites around all the teeth (six sites around each tooth). Such probing involves the insertion of a thin metal instrument (periodontal probe), which is advanced to the base of the pocket until resistance is met by the first intact junctional epithelium and collagen fibers [9]. Measurements obtained by this method are variable, owing to several factors that can lead to underestimation or overestimation of PP depth, including intra-and-inter examiner differences of pressure and/or angulation exerted during insertion of the periodontal probe, and the presence or absence of inflammation and/or sub-gingival calculus [10–17]. This method is also time consuming, sometimes uncomfortable to patients, and can elicit a low grade bacteremia [18,19]. Periodontal probing yields a "linear" estimate of the PP and is not suitable for evaluating spiral or complex types of PP that require 3-D considerations. Clearly, PP analyses need to be updated using more advanced digital 3-D imaging technology, such as cone beam computed tomography (CBCT). Unfortunately, such X-ray-based analyses are currently incapable of imaging soft tissue defects because of its low resolution for soft tissue [20]. Consequently, the use of a biocompatible radiopaque contrast agent to temporarily fill the PP could enable precise 3-D imaging of the PP with the CBCT.

The objectives of this study are: (1) to determine the feasibility of using various radiopaque micro-particle filler formulations to enable high-resolution, 3-D imaging by micro-computed tomography (μCT) of simulated periodontal pockets, (2) to investigate the biocompatibility/toxicity of such fillers, and (3) to evaluate whether an additional antibacterial capability can be introduced to such fillers *via* the incorporation of a quaternary ammonium compound (QAC).

2. Materials and methods

2.1. Characterization of metal oxide-bearing micro-particle filler formulations for PP imaging

Standard filler formulations examined in this project were composed of mixtures comprised of 40 wt% metal oxide micro-particles in 60 wt% glycerol. Formulations containing the following five different types of calcium oxide-bearing and/or titanium oxide-bearing particles were examined: calcium tungstate, $CaWO_4$; calcium zirconate, $CaZrO_3$; calcium titanate, $CaTiO_3$; titanium oxide, TiO_2; and hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. These mixtures were evaluated for use as temporary fillers in PP analogues (*i.e.*, for placement in the PP analogues for durations just long enough to obtain digital X-ray images of the PP). The oxide micro-particles were obtained from a commercial source (−325 mesh, <44 μm diameter, Sigma Chemical Co, St. Louis, Mo).

2.2. MicroCT (μCT) imaging of micro-pipette and tooth/gingiva analogue models containing radiopaque micro-particle-based fillers

The sensitivity and resolution (6–30 μm voxels) of a micro-computed tomography (μCT) system (Skyscan 1174; Micro photonics Inc; PA, USA) facilitated *in vitro* testing of the micro-particle fillers. Initial scanning was conducted at 50 kV and 800 μA. The images were reconstructed using μCT software (Skyscan NRecon/NRecon server software Micro photonics Inc; PA, USA). Two experimental *in vitro* models were designed to evaluate the relative radiopacities of formulations containing various metal oxide micro-particles (including hydroxyapatite, HA). The first was a simple micro-pipette model (Fig. 1A) in which micro-particle fillers were placed in a long micro-pipette tip possessing an internal diameter (ID) ranging from 0.2 mm (at the bottom end) to 0.5 mm (at the upper end), and then imaged by μCT (we have estimated that thickness of the periodontal pocket apically could be as low as that of

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