Journal of Dentistry 43 (2015) 1462–1469

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.intl.elsevierhealth.com/journals/jden

Anti-biofilm activity of zinc oxide and hydroxyapatite nanoparticles as dental implant coating materials

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ARTICLE INFO

Article history:
Received 3 June 2015
Received in revised form 28 September 2015
Accepted 15 October 2015

Keywords:
Nanoparticles
Peri-implantitis
Zinc oxide
Coatings
Biofilm
Antimicrobial

ABSTRACT

Objectives: Dental implants are prone to failure as a result of bacterial biofilm accumulation. Such biofilms are often resistant to traditional antimicrobials and the use of nanoparticles as implant coatings may offer a means to control infection over a prolonged period. The objective of this study was to determine the antibiofilm activity of nanoparticulate coated titanium (Ti) discs using a film fermenter based system.
Methods: Metal oxide nanoparticles of zinc oxide (nZnO), hydroxyapatite (nHA) and a combination (nZnO + nHA) were coated using electrohydrodynamic deposition onto Ti discs. Using human saliva as an inoculum, biofilms were grown on coated discs for 96 h in a constant depth film fermenter under aerobic conditions with artificial saliva and peri-implant sulcular fluid. Viability assays and biofilm thickness measurements were used to assess antimicrobial activity.
Results: Following 96 h, reduced numbers of facultatively anaerobic and *Streptococcus* spp. on all three nano-coated surfaces were demonstrated. The proportion of non-viable microorganisms was shown to be higher on nZnO and composite (nZnO + nHA) coated surfaces at 96 h compared with nHA coated and uncoated titanium. Biofilm thickness comparison also demonstrated that nZnO and composite coatings to be the most effective.
Conclusions: The findings support the use of coating Ti dental implant surfaces with nZnO to provide an antimicrobial function.
Clinical significance: Current forms of treatment for implant associated infection are often inadequate and may result in chronic infection requiring implant removal and resective/regenerative procedures to restore and reshape supporting tissue. The use of metal oxide nanoparticles to coat implants could provide osteoconductive and antimicrobial functionalities to prevent failure.

1. Introduction

Despite measures taken to prevent bacterial infection, complications with dental implants persist including those associated with antimicrobial treatment [1,2]. Upon contact, bacteria adhere, multiply and form a biofilm on the untreated surface of an implant [3]. To help overcome this issue, implants can either be coated or impregnated with antimicrobial agents. However, many such approaches have been unsuccessful and resistance to those antimicrobials employed is often observed. In these cases, it is the complex structure of the associated microbial community that makes it difficult for antimicrobial agents to penetrate [4]. Resistance can also involve other mechanisms [5], including microbial efflux systems that pump antimicrobial agents out of the cell [6] and the production of exopolymers which may additionally prevent penetration of agents and thus lead to the persistence of the microbial community [7]. Failure or loosening of an osseointegrated implant will allow for further microbial growth, possibly resulting in peri-implantitis with associated soft and hard tissue damage [8]. While the microbiota associated with oral health, including that around implants consists of predominantly Gram positive spp., that associated with oral infection and infected implants is predominantly Gram negative and includes *Porphyromonas gingivalis*,*Fusobacterium* spp. and *Treponema denticola* [9]. The presence of such pathogenic bacteria in the oral

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http://dx.doi.org/10.1016/j.jdent.2015.10.010
0300-5712/

cavity and other biofilms has led to the development of prophylactic approaches that involve the use of single or multiple antibiotics [10]. However, such antibiotic treatments provide opportunities for resistance to develop when inappropriate doses are used to prevent biofilm formation [11–13].

In particular, antibiotics with a single target will encourage resistance and therefore reduced efficacy of the drug. There is thus a need to investigate alternative approaches as opposed to the use of traditional antimicrobial agents [9]. This may be possible by exploitation of the antimicrobial ability of metal based antimicrobial nanoparticles (NPs) to control biofilm formation. Their unique physiochemical properties should help avoid antimicrobial resistance, as activity is thought to involve multiple targets thus hindering rapid microbial adaptation and evolution [10–12].

Metallic NPs possess bactericidal effects against a variety of species [13], withstand high temperatures required for fixation and possess low toxicity to mammalian cells when stabilised onto surfaces [14,15]. In order to utilise such antimicrobial capability, appropriate coating methods are required. Generally, coatings used for dental implants employ extreme conditions such as very high temperatures that can alter the properties of the coatings or surfaces [16]. These conditions are often intended for stability and biological enhancement of the implant's surface, however with respect to NPs, it is more suitable to use lower temperatures to retain beneficial physiochemical properties. Electrohydrodynamic atomisation (EHDA), a simple and economical spraying technique allows for the implementation of such conditions [17]. This method produces a uniform and stable coating whilst covering a large area and so enables sufficient functionality without compromising the original state of the coating [17]. Zinc oxide NPs (nZnO) are currently being investigated for their antimicrobial activity and show much promise [18,19]. Studies suggest that the antimicrobial effects of these NPs is due to ion release or via the production of reactive oxygen species (ROS) [20]. It is likely that the antimicrobial properties of NPs will differ according to their shape, surface area, size and relative chemical properties and thus it is these characteristics that lead to the overall synergistic activity [21]. Recent studies indicate that the utilisation of nZnO also allows for the promotion of bone growth in addition to enhanced osteoblast proliferation. [22,23]. Furthermore, investigations involving HA as a coating material indicate a stable interface can be formed between bone and implant [24]. With respect to dentistry, calcium phosphates have played a key role due to their physical and chemical similarities to bone and mineralised tissues, such as enamel and dentine [25]. Hence, nano-scale HA (nHA) closely resembles the size and properties of HA crystals in natural bone [26]. Furthermore, studies suggest that nHA has the ability to inhibit the growth of both Gram-negative and Gram-positive bacteria, including *Staphylococcus aureus* and *Staphylococcus epidermidis* [27].

The current investigation was performed using a constant depth film fermenter (CDFF) in order to improve our understanding of the antimicrobial activity of nZnO, nHA and their composite. It is hypothesized that such coating materials will be able to prevent infection as a cause of early or late dental implant failure.

2. Materials and methods

2.1. Nanoparticles

The NPs tested were: nZnO, nHA and a combination of 50% nZnO and 50% nHA. Nano-ZnO particulates were synthesised using flame pyrolysis as carried out by Johnson Matthey plc (JMTC) and nHA were synthesised as previously described [28]. The surface area for nZnO was determined by Brunauer Emmett Teller (BET) analysis at JMTC.

2.2. Ti samples

Grade 23 moderate smooth machined Ti discs (Hyundai Hit 8S—A C Service Group, Fordingbridge, UK) 5 mm in diameter and 2 mm in height were used in all experiments. The Ti discs were sonicated with acetone for 20 min at 50 Hz in order to remove adherent debris or dust and then autoclaved before coating.

2.3. EHDA spraying

Nano-HA, nZnO and their combination of equal proportions were used as suspensions for electrohydrodynamic spraying. This method uses a fine jet containing NPs which are deposited onto the surface of substrates. In the present study, NPs were suspended in 100% ethanol and jetted via a needle under an electric field. A stainless steel needle with a diameter of 300 μm was used to spray the content of a 1 mL syringe onto the surface of the sample. The outcome is a symmetrical cone shaped jet spray which delivers an optimal coating to ensure coverage of the sample. The distance between the substrate and the needle was maintained at 30 mm at all times. All experiments were performed with a freshly prepared suspension (10,000 μg/mL) of NPs. The voltage used was between 4.5–5 kV with Ti discs coated for a period of 1 min at a flow rate of 5 μL/min. All coated and uncoated samples were subjected to heat treatment (600 °C) for 1 h. Samples were gradually cooled to maintain mechanical integrity of the coating (1 °C/min).

2.4. Saliva samples

In order to produce microcosm biofilms, saliva (unstimulated) was collected aseptically from ten members of the Department of Microbial Diseases, Eastman Dental Institute, UCL. These individuals had no significant oral disease (Ethical approval obtained from the UCL Ethics Committee; Project no. 1364/001). The pooled samples were resuspended in 10% (v/v) glycerol and vortex mixed vigorously for 1 min to homogenise. Aliquots of 1 mL were then dispensed and stored at −80 °C for subsequent use.

2.5. Constant depth film fermenter

A vial of stored pooled saliva was thawed and 900 μL was used to inoculate 500 mL of artificial saliva. To make 1 L of artificial saliva 0.5 g 'Lab-Lemco' powder (Oxoid, UK), 1 g yeast extract (Oxoid, UK), 2.5 g protease peptone (Oxoid, UK), 1.25 g hog gastric mucin type III, partially purified from porcine stomach (Sigma Chemicals Co., Poole, UK), 0.18 g sodium chloride (BDH Chemicals Ltd, Poole, UK), 0.1 g calcium chloride (BDH) and 0.1 g potassium chloride (BDH) was added to deionised water. After autoclaving at 121 °C, 0.625 mL of a 40% urea (Sigma–Aldrich) solution was added. This was mixed and pumped into the CDFF for 6 h at a rate 1.38 mL/min. The CDFF [29,30] was used to grow microcosm biofilms on nano-coated and uncoated Ti samples. Briefly, the CDFF apparatus consists of a glass vessel with a stainless steel top endplate that allows for entry of nutrient medium, gas and sampling in addition to a bottom endplate designed for medium outlet. Subsequently, the inoculation flask was disconnected and the CDFF fed from a medium reservoir of sterile artificial saliva and peri-implant sulcular fluid (40 μL/mL). This was delivered via a peristaltic pump (Watson–Marlow, Falmouth, UK) at a rate of 0.5 mL/min (0.72 L/day). The experiments were performed using two separate runs. Coated and uncoated Ti samples were positioned on PTFE plugs (located within a rotating turntable), held in place using vacuum silicone grease (Dow Corning) and recessed to a depth of 600 μm. These experiments were designed to mimic the conditions associated with a dental implant under aerobic conditions for 5 days (37 °C).