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In vivo bone response and mechanical evaluation of electrosprayed CaP nanoparticle coatings using the iliac crest of goats as an implantation model

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ABSTRACT

Recent trends in clinical implantology include the use of endosseous dental implant surfaces embellished with nano-sized modifications. The current study was initiated to evaluate the mechanical properties, as well as the potential beneficial effects, of electrosprayed CaP nanoparticle-coated (nano-CaP) implants on the in vivo osteogenic response, compared with grit-blasted, acid-etched (GAE) implant surfaces as controls. For this purpose nano-CaP coatings were deposited on cylindrical screw-type (St) implants and implanted bilaterally into the iliac crest of goats for 6 weeks. In addition to histological and histomorphometrical analyses, insertion torque and removal torque values were measured on implant placement and retrieval, respectively. The present study showed similar insertion and removal torque values for nano-CaP-coated and GAE control implants, with no statistically significant increase in torque value during the implant period for either group. With regard to bone–implant contact and peri-implant bone volume, no significant differences were found between nano-CaP-coated and GAE implants after 6 weeks implantation. In conclusion, this study has demonstrated that in situations in which implants are placed in a non-compromised situation using a standard press fit implantation strategy the performance of electrosprayed nano-CaP coatings is comparable with GAE implants, both with respect to implant fixation and bone healing response.

1. Introduction

The long-term clinical success of orthopedic and dental implants is greatly influenced by their physicochemical surface characteristics, since the overall tissue response (i.e. adsorption of proteins, cell adhesion and spreading) responsible for optimal anchoring of the implant into the native bone tissue, takes place at the surface of the implant [1,2]. Consequently, research is increasingly focusing on the modification of implant surfaces to improve the properties of the bulk material and thereby enhance the biological healing response. These implant surface modifications can rely on either chemical or topographical alterations, or a combination thereof [3].

Various reports have already claimed a positive correlation between implant micro/nano surface roughness and interfacial strength, which can, for example, be measured by removal torque testing [4–6]. In addition, a faster rate and higher degree of bone formation has been reported for roughened surfaces [7–10]. Regarding surface chemistry, bioactive materials, such as calcium phosphate (CaP) ceramics, have routinely been applied as thin coatings onto metallic implant materials (mostly titanium, Ti), as these ceramics are too brittle for use as a bulk material under loaded conditions. The excellent biological properties of CaP ceramics can be exploited in combination with the mechanical strength of metallic materials in such coated implants [11–14].

Since the introduction of CaP coatings these ceramics have proved to be osteoconductive [15,16], to improve implant fixation [17], to increase bone–implant contact [18,19] and to facilitate the bridging of gaps up to 1.0 mm [20,21]. Various techniques have been used to deposit CaP coatings onto implant surfaces, of which plasma spraying is still the most widely used [14,22,23]. Despite positive results regarding the osteoconductive and bone bonding behavior of plasma-sprayed coatings [24,25], this deposition technique is only capable of producing coatings with a minimal thickness of 30 μm, thereby introducing the risk of coating delamination. Additionally, incorporation of organic biomolecules (e.g. growth factors) to further enhance the biological activity of CaP coatings has been hampered due to the extremely high temperatures during the plasma spray process. To overcome

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this limitation the electrospraying of suspensions of nano-sized crystalline CaP particles was recently suggested, to enable the deposition of nanometer thin CaP films at low temperatures [26]. As in these suspensions CaP crystals have already been formed prior to spraying, high temperatures during coating deposition and additional heat treatments to crystallize the ceramic are bypassed. Moreover, nanometer thin coatings will reduce the risk of coating delamination related to the micrometer thick plasma-sprayed coatings. Also, from a biological point of view there is increasing interest in the use of CaP nanoparticles (nano-CaP) for orthopedic and dental applications, as they resemble the nano-crystalline nature of bone mineral, increase osteoblast adhesion [27] and improve osteogenic behavior [28]. To date, it has been shown that CaP particle size is directly related to the bioactive properties of CaP [29,30]. Although these results are promising, in vivo experiments are needed to obtain conclusive data on the capacity of nanometer thick electrosprayed CaP coatings to enhance the osteogenic response.

Consequently, the current study aimed at evaluating the mechanical properties and in vivo response of electrosprayed nano-CaP-coated implants using a goat implantation model. For this purpose, nano-CaP coatings were deposited onto cylindrical screw-type (St) implants and implanted bilaterally into the iliac crest of goats for 6 weeks. Grit-blasted, acid-etched (GAE) implants served as controls. Insertion and removal torque values were determined for the bone implants at implant placement and retrieval, respectively. Further, the osteogenic response was evaluated qualitatively (histology) and quantitatively (histomorphometry).

2. Materials and methods

2.1. Materials

Helix® dental implants, made of titanium alloy (Ti–6AL–4V), grit-blasted and acid-etched (GAE) to roughen the surface (roughness R_a = 1.3–1.4 μm), were provided by Dyna Dental Engineering BV (Bergen op Zoom, The Netherlands). These cylindrical St implants were based on a root shape core and a straight, self-tapping thread measuring 13 mm in length and 4.2 mm in diameter. Commercially available nano-CaP suspensions were acquired from Berkeley Advanced Biomaterials (Berkeley, CA).

2.2. Implant preparation and cleaning

Prior to coating deposition all St implants were cleaned ultrasonically in 10% nitric acid (15 min), acetone (15 min) and isopropanol (15 min) and thereafter air dried.

2.3. Coating deposition

For coating deposition at low temperatures nano-CaP suspensions containing nano-sized crystalline carbonate apatite particles were used, as reported previously [28]. Commercially available ethanol-based CaP suspensions (Berkeley Advanced Biomaterials) were diluted in 100% ethanol (10:90 vol.%) prior to electrospraying. To deposit the nano-CaP coatings a commercially available vertical electrostatic spray deposition set-up (Advanced Surface Technology, Bleiswijk, The Netherlands), as described by de Jonge et al. [31], was used. The St implants were coated in two runs (with turns of 180°) of 5 min each to obtain complete coverage. The standardized conditions were: 15% relative humidity; 40 °C substrate holder temperature; 40 mm nozzle to substrate distance; 0.15 ml h^{-1} liquid flow rate; 8–10.5 kV applied voltage.

2.4. Coating characterization

2.4.1. Coating thickness

The thickness of the deposited nano-CaP coating, corresponding to an electrospray deposition time of 5 min, was determined by atomic force microscopy (AFM) (Multimode Nanoscope IIIa) in an accurate model system using silicon wafers as substrate (unpublished results). Briefly, one half of the silicon wafers was coated with nano-CaP while the other half was left uncoated. Subsequently the wafers were scanned at the non-coated/coated boundary in tapping mode at a rate of ~1 Hz using 100 μm long silicon cantilevers (NSG10, NT-MDT) with average nominal resonant frequencies of 250 kHz, spring constants of 15 N m^{-1} and a tip radius of curvature of <10 nm. To analyze the height difference between the silicon wafer and the coating, which corresponds to the coating thickness, nanoscope imaging software (version 6.13rl, Veeco) was used.

2.4.2. Coating morphology

Scanning electron microscopy (SEM) (JEOL 6310, Tokyo, Japan) was performed to examine the surface morphology of the implants of both experimental groups (GAE and nano-CaP).

2.4.3. Coating surface roughness

Average surface roughness values (R_a) were determined for both experimental groups (GAE and nano-CaP) using a Universal Surface Tester (UST) (Innowep, Wurzburg, Germany).

2.4.4. Coating adhesion upon implant insertion in artificial bone

To evaluate adhesion of the nano-CaP coatings to the St implants biomedical test blocks (Sawbones®; Pacific Research Laboratories, Washington, DC) were used as an artificial bone model. These test blocks offer uniform and consistent mechanical properties that eliminate the variability encountered when using cadaver bones. The test blocks consisted of solid, rigid polyurethane foam with a density of 0.48 g cm^{-3} covered with a 1 mm thick fiber filled epoxy sheet, corresponding to human cancellous and cortical bone, respectively. In accordance with the manufacturer's recommendations, drill holes with a diameter of 4.0 mm were made in the test blocks using a dental drill (KaVo EWL Dental GmbH, Biberach, Germany) at a bit speed of 2000 r.p.m. under continuous cooling. Subsequently the St implants were installed. In order to prevent damage to the coating upon explantation by means of unscrewing the test blocks were cross-sectioned and the implants removed. In this way the test procedure closely resembles the clinical situation, in which implants are left in situ after installation. After explantation the implants were carefully brushed to remove adherent polyurethane foam fragments. Subsequently the nano-CaP coatings were thoroughly inspected using SEM. The amount of nano-CaP coating remaining on the implant surface was quantified using the ortho-cresolphthalein complexone (OCPC) method. In brief, the implants were incubated overnight in 1 ml of 0.5 N acetic acid on a shaker table. For analysis 300 μl of work reagent was added to aliquots of 10 μl of sample or standard in a 96-well plate. The plate was incubated for 10 min at room temperature, after which the plate was read at 570 nm. Serial dilutions of $CaCl_2$ (0–100 μg ml^{-1}) were used to produce a standard curve. As received nano-CaP-coated implants were used as controls.

2.5. Experimental animal groups

In the present study St implants were placed in the iliac crests of four goats. Two experimental groups were used: grit-blasted and acid-etched (GAE); GAE + nano-CaP. Sterility of the substrates was obtained by autoclaving.