



(51) International Patent Classification:

A61L 27/30 (2006.01) *A61L 27/14* (2006.01)
A61L 27/04 (2006.01) *C25D 15/00* (2006.01)
A61L 27/10 (2006.01)

(21) International Application Number:

PCT/US2016/055683

(22) International Filing Date:

6 October 2016 (06.10.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/237,848 6 October 2015 (06.10.2015) US

(71) Applicants: UNIVERSITY OF CENTRAL FLORIDA RESEARCH FOUNDATION, INC. [US/US]; 12201 Research Parkway, Suite 501, Orlando, FL 32826 (US). SANFORD BURNHAM PREBYS MEDICAL DISCOVERY INSTITUTE [—/US]; 10901 North Torrey Pines Road, La Jolla, CA 92037 (US).

(72) Inventors: SEAL, Sudipta; 13139 Hatherton Circle, Orlando, FL 32832 (US). DAS, Soumen; 12001 Solon Drive, Apt. 202, Orlando, FL 32826 (US). SELF, William; 3600 Foxcroft Circle, Oviedo, FL 32765 (US). TOWLER, Dwight; 10901 North Torrey Pines Road, La Jolla, CA 92037 (US).

(74) Agents: VAN DYKE, Timothy, H. et al.; Beusse, Wolter, Sanks & Maire, PLLC, 390 N. Orange Ave., Suite 2500, Orlando, FL 32801 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: IMPLANT AND COATING TO REDUCE OSTEOLYSIS

(57) Abstract: An implant is provided comprising a substrate having one or more nanoceria coatings coated at least partially thereon, wherein the one or more nanoceria coatings comprise surface cerium having a 3+/4+ oxidation state ratio such that the one or more nanoceria coatings exhibit catalase mimetic activity, superoxide dismutase mimetic activity, or both. Methods are provided for forming a nanoceria coating. The coating has nanoceria having a surface cerium 3+/4+ oxidation state ratio such that the coating exhibits catalase mimetic activity, superoxide dismutase mimetic activity, or both. Also disclosed is a method of reducing degradation of an implant by placing nanoceria in proximity to a bone-implant interface.



IMPLANT AND COATING TO REDUCE OSTEOLYSIS

Background

The American Academy of Orthopedic Surgeons has projected annual volumes of primary total hip joint replacement to increase over 748,000 in USA or 4 million worldwide by 2030. By 2030, total hip replacement surgery is expected to rise by 174% and total knee replacement by 673%. Despite technological advances and improvements in treatment strategies to manage rheumatoid arthritis and osteoarthritis, total joint replacement (“TJR”) still remains the final treatment option in many cases to relieve pain, and improve the quality of life. Annual hospital costs associated with these procedures are projected to exceed \$65 billion by 2015. However, due to osteolysis 10-15% TJR will fail, with some studies suggesting rates of osteolysis can approach 40%.

In the USA, the annual cost of TJR exceeds \$10 billion. Although *joint replacement* surgery has made remarkable progress, 10-15% arthroplasty failure still occurs due to high levels of free radicals, chronic inflammation and osteolysis. In 2000, 28,000 and 31,000 revision surgeries were performed for total hip arthroplasty and total knee arthroplasty, respectively and the numbers of revision surgeries are increasing each year. Revision surgeries are 40% more costly than primary total hip and knee arthroplasties and more than 1 billion dollars are spent on revision surgeries each year alone in USA. At this time, there is no drug or treatment strategies specifically approved for prevention or inhibition of periprosthetic osteolysis (hereinafter “osteolysis”).

Cerium is a rare-earth element with fluorite lattice structure with +3/+4 oxidation states and may interchange between the two depending on the environment. Cerium oxide nanoparticles (hereinafter “CNP”s) have been shown to possess a substantial oxygen storage capacity via the interchangeable surface reduction and oxidation of cerium atoms, cycling between the Ce^{4+} and Ce^{3+} redox states. CNPs have a mixed oxidation state of cerium

containing both Ce^{3+} and Ce^{4+} . It has been shown that upon incubation of CNPs with hydrogen peroxide, CNPs with a higher starting concentration of Ce^{3+} can convert to CNPs containing increased Ce^{4+} on their surface. Along with this change in oxidation state is the loss of their SOD mimetic ability. However, increased Ce^{4+} on the CNP surface exhibit better
5 catalase mimetic and $\cdot\text{NO}$ scavenging capabilities.

It has been well established in many studies that depending on their reactivity and surface chemistry, CNPs can effectively convert both reactive oxygen species (ROS)(superoxide, $\text{O}_2^{\cdot-}$, and hydrogen peroxide) into more inert species and scavenge reactive nitrogen species (RNS)(nitric oxide, $\cdot\text{NO}$), both *in vitro* and *in vivo*. It has been
10 further shown that CNPs significantly accelerate the decay of ONOO^- and that CNPs ability to interact with ONOO^- is independent of the $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratio on the surface of the CNPs.

Due to these capabilities, these materials have been employed for industrial use in three-way catalysts. Biological uses of CNPs have centered on their ability to scavenge free radicals under physiologically relevant conditions. This catalytic nature, which began with
15 the discovery that water-based CNPs (with increased Ce^{3+} in their outer surface) could act as superoxide dismutase mimetics, has laid the foundation for their application in experimental and biomedical research.

CNPs are known for their regenerative antioxidant activity in a biological environment. The unique regenerative property of CNPs is due to low reduction potential and
20 the existence of both $\text{Ce}^{3+}/\text{Ce}^{4+}$ oxidation states. It has been shown that the oxygen vacancies could act as catalytically active hot spots to scavenge very reactive radicals such as superoxide radical anion, hydrogen peroxide, nitric oxide or peroxynitrite. It has been shown that NC with higher levels of cerium in the +3 oxidation state exhibit superoxide dismutase activity and that this reactivity correlates with the level of cerium in the +3 oxidation state in
25 a reversible manner. Likewise, NC with higher levels of surface cerium in the +4 oxidation

state exhibit better catalase mimetic activity that also is reduced when higher levels of cerium are present in the +3 oxidation state.

Brief Description of the Drawings

Figure 1 is a schematic showing the mechanisms by which a NC coating can prevent osteolysis by scavenging free radicals, suppressing immune reaction locally and inhibiting corrosion, in accordance with an embodiment.

Figure 2 shows topology and catalytic properties of the NC coating. (A) Surface morphology of EPD coated NC (left – pulse EPD; right – direct current) in accordance with an embodiment; B) Real time degradation of H_2O_2 followed absorbance at 240 nm; (C) ROS estimated using H_2DCF fluorescence in an NC coated substrate in accordance with an embodiment; and (D) RNS using APF fluorescence assay in an NC coated substrate, in accordance with an embodiment.

Figure 3 shows (A) HRTEM images of NC1. 3-5 nm NC1; (B) the interplanar spacing of lattice, representation of fluorite structure of NC (selected area diffraction pattern). (C) XPS spectra show variation in Ce^{3+}/Ce^{4+} in two different NC (solid line NC1; sphere–NC2).

Figure 4 depicts RAW cells grown for 18hr on the surface of 3 min and 6 min EPD coated Ti substrate.

Figure 5 shows matrix metalloproteinases, MMP9, MMP12 and MMP14 expression in a nanoceria coated substrate after induction with RANKL OR TNF.

Figure 6 shows a measure of cell proliferation on a coated Ti-metal substrate at different roughnesses, in accordance with an embodiment.

Figure 7 shows primary hank osteoblasts were plated onto type I collagen-coated (A) control or (B) NC-coated Ti plates & cultured for 8 days. Alkaline phosphatase activity was visualized by Vector Red staining, in accordance with an embodiment.

Figure 8A shows an electric field simulation in a two v. three electrode configuration.

5 **Figure 8B** is a graph showing the coating Ce^{3+}/Ce^{4+} ratio as a function of electrode distance and time, in accordance with an embodiment.

Figure 9 are photos showing a comparison showing RAW cell growth on EPD coated Ti-substrate and a control Ti (uncoated).

10 **Figure 10** is a graph showing NF κ B-LUC Activity in RAW osteoclast progenitors (TK Renilla Normalized).

Figure 11A shows the intensity of the electric field of the electrodes obtained by finite element modeling using COMSOL.

Figure 11B shows the open circuit potential (OCP) of uncoated and NC coated sample substrates.

15 **Figure 12** shows a flow chart for *in vivo* testing to confirm anti-inflammatory properties, osseointegration, and mechanical testing of coatings, in accordance with embodiments.

20 **Detailed Description**

Total joint replacement and the use of prostheses/implants are rapidly growing due to higher life expectancy and the growing obesity epidemic. However, 10-15% of implants will

fail and will need extensive revision surgery mainly due to osteolysis. At this time, there are no drug or treatment strategies specifically approved for prevention or inhibition of periprosthetic osteolysis.

It was previously unknown whether coating forms of CNPs would exhibit the same catalytic activity described above. One concern was the ability to create a coating which retained the catalytic and radical scavenging properties described above and in the cited references, as previous studies involved CNPs. In contrast, a nanoceria ("NC") coating is provided herein that is formed on a substrate (or on an intermediate layer between the substrate and the coating) and as such, no longer in nanoparticle form. It is shown through experiments described below the NC coatings developed and disclosed retain the catalytic mimetic activities reported for the CNPs. Moreover the NC coatings enhance osseointegration, and reduce overall osteolysis. Both methods and products are disclosed.

As to the methods, embodiments disclosed include making an implant comprising the steps of obtaining a substrate comprising materials comprised in whole or in part of metal, ceramic, plastic, or a composite; and depositing a nanoceria coating on at least a portion of the substrate, wherein the nanoceria coating has a predetermined surface roughness parameter and a surface cerium 3+/4+ oxidation state ratio such that said nanoceria coating exhibits catalase mimetic activity, superoxide dismutase mimetic activity, or both. In these and other embodiments, the implant is adapted for the prevention or inhibition of osteolysis.

Embodiments described herein are based on the discovery that a significant cause of arthroplasty failure occurs due to high levels of free radicals, chronic inflammation and osteolysis. Osteolysis is the destruction of bone tissue. This may occur due to chronic inflammation from particles or debris generated through wear, electrochemical dissolution/corrosion, or a combination thereof. The implant coated at least partially thereon with an NC coating is able to reduce the presence of such free radicals and thus overall

inflammation and osteolysis. Therefore, a method is also disclosed for reducing degradation of an implant by placing nanoceria in proximity to a bone-implant interface.

Further embodiments include an implant, or component of an implant, having said NC coating. As used herein, the term “component” refers to a part of an overall implant, bone
5 implant, or other prosthesis. These implants comprise a NC coating on at least a portion thereof. In such an embodiment, the implant comprises at least a substrate on which an NC coating is coated; there may also be an intermediate layer between the coating and the substrate. Also disclosed are embodiments relating to a coating itself, without respect to any substrate or intervening layer upon which the coating may be disposed. The NC coatings of
10 embodiments have various features. Namely, the coating is characterized by nanoceria having a surface cerium 3+/4+ oxidation state ratio such that the coating exhibits catalase mimetic activity, superoxide dismutase mimetic activity, or both.

Because cell attachment and proliferation of macrophage cells (involved in the osteolytic process) were shown to be inversely proportional with increasing roughness of the
15 coating, the coatings disclosed may also have a predetermined surface roughness (described below). Methods for electrodeposition of an NC coating on a substrate are also disclosed. Broadly, a method comprises electrophoretically forming the coating on a given substrate using a dispersion of CNPs, preferably where the substrate is placed between two counter electrodes (for a total of three electrodes). However, a two electrode setup may also be used.
20 This coating method uses CNPs having surface cerium in the 3+ oxidation state or 4+ oxidation state, or both, and results in an NC coating which has a given 3+/4+ ratio such that the desired and above described anti-inflammatory properties are exhibited. Coatings with both oxidation states are preferable, but either oxidation state imparts advantageous benefits.

According to another embodiment, a method is provided for conducting an orthopedic
25 procedure in a subject in need thereof. The method involves obtaining an implant having an

NC coating as described herein and implanting the implant into the subject at a site of need. The implantation involves positioning the implant so as to have contact with bone tissue. In a specific embodiment, the orthopedic procedure is an arthroplasty procedure, and the implant is one for insertion in or between a joint. Examples of arthroplasty implants include
5 hip replacement implants, knee replacement implants, shoulder replacement, and intervertebral implants. Other related orthopedic implants that may be coated and used in accordance with the embodiments described herein include, but are not limited, plates, screws, rods, cages, dowels and the like used in orthopedic surgeries. A subject as used herein refers to a mammal including but not limited to a human, dog, cat, horse, goat, cow
10 etc. A subject in need is one who has an injury, defect or disease of the musculoskeletal system requiring an orthopedic surgery.

Furthermore, based on the discoveries herein, an alternative embodiment relates to a bone paste composition that includes CNPs comprising catalase activity, superoxide dismutase activity, or both, and at least one osteoinductive or osteoconductive component.
15 Examples of osteoinductive or osteoconductive components include, but are not limited to, demineralized bone powder as described in U.S. Pat. No. 5,073,373 the contents of which are incorporated herein by reference, collagen, insoluble collagen derivatives, hydroxyapatite, ceramic, calcium phosphate, dicalcium phosphate, tricalcium phosphate, bone morphogenetic protein, transforming growth factor (TGF-beta), insulin-like growth factor (IGF-1) (IGF-2),
20 platelet derived growth factor (PDGF), fibroblast growth factors (FGF), vascular endothelial growth factor (VEGF), angiogenic agents, bone promoters, cytokines, interleukins, genetic material, genes encoding bone promoting action, cells containing genes encoding bone promoting action; growth hormones such as somatotropin; bone digestors; antitumor agents; fibronectin; cellular attractants and attachment agents. US patent

6,695,882, incorporated by reference, teaches other osteoinductive and osteoconductive components.

In a further embodiment, the bone paste includes a suitable carrier component. Examples of carrier components include, but are not limited to:

- 5 (i) Polyhydroxy compound, for example, such classes of compounds as the acyclic polyhydric alcohols, non-reducing sugars, sugar alcohols, sugar acids, monosaccharides, disaccharides, water-soluble or water dispersible oligosaccharides, polysaccharides and known derivatives of the foregoing. Specific polyhydroxy compounds include, 1,2-propanediol, glycerol, 1,4,-butylene glycol trimethylolethane, trimethylolpropane, erythritol, 10 pentaerythritol, ethylene glycols, diethylene glycol, triethylene glycol, tetraethylene glycol, propylene glycol, dipropylene glycol; polyoxyethylene-polyoxypropylene copolymer, e.g., of the type known and commercially available under the trade names Pluronic and Emkalyx; polyoxyethylene-polyoxypropylene block copolymer, e.g., of the type known and commercially available under the trade name Poloxamer; 15 alkylphenolhydroxypolyoxyethylene, e.g., of the type known and commercially available under the trade name Triton, polyoxyalkylene glycols such as the polyethylene glycols, xylitol, sorbitol, mannitol, dulcitol, arabinose, xylose, ribose, adonitol, arabitol, inositol, fructose, galactose, glucose, mannose, sorbose, sucrose, maltose, lactose, maltitol, lactitol, stachyose, maltopentaose, cyclomaltohexaose, carrageenan, agar, dextran, alginic acid, guar 20 gum, gum tragacanth, locust bean gum, gum arabic, xanthan gum, amylose, mixtures of any of the foregoing, and the like.

- (ii) Polyhydroxy ester, for example, liquid and solid monoesters and diesters of glycerol can be used to good effect, the solid esters being dissolved up to the limit of their solubilities in a suitable vehicle, e.g., propylene glycol, glycerol, polyethylene glycol of 200- 25 1000 molecular weight, etc. Liquid glycerol esters include monacetin and diacetin and solid

glycerol esters include such fatty acid monoesters of glycerol as glycerol monolaurate, glyceryl monopalmitate, glyceryl monostearate, etc. An especially preferred carrier herein comprises glyceryl monolaurate dissolved in glycerol or a 4:1 to 1:4 weight mixture of glycerol and propylene glycol, poly(oxyalkylene) glycol ester, and the like.

5 (iii) Fatty alcohol, for example primary alcohols, usually straight chain having from 6 to 13 carbon atoms, including caproic alcohol, caprylic alcohol, undecyl alcohol, lauryl alcohol, and tridecanol.

(iv) Fatty alcohol ester, for example, ethyl hexyl palmitate, isodecyl neopentate, octadodecyl benzoate, diethyl hexyl maleate, and the like.

10 (v) Fatty acid having from 6 to 11 carbon atoms, for example, hexanoic acid, heptanoic acid, octanoic acid, decanoic acid and undecanoic acid.

(vi) Fatty acid ester, for example, polyoxyethylene-sorbitan-fatty acid esters; e.g., mono- and tri-lauryl, palmityl, stearyl, and oleyl esters; e.g., of the type available under the trade name Tween from Imperial Chemical Industries; polyoxyethylene fatty acid esters; e.g.,
15 polyoxyethylene stearic acid esters of the type known and commercially available under the trade name Myrj; propylene glycol mono- and di-fatty acid esters such as propylene glycol dicaprylate; propylene glycol dilaurate, propylene glycol hydroxy stearate, propylene glycol isostearate, propylene glycol laureate, propylene glycol ricinoleate, propylene glycol stearate, and propylene glycol caprylic-capric acid diester available under the trade name Miglyol;
20 mono-, di-, and mono/di-glycerides, such as the esterification products of caprylic or caproic acid with glycerol; e.g., of the type known and commercially available under the trade name Imwitor; sorbitan fatty acid esters, e.g., of the type known and commercially available under the trade name Span, including sorbitan-monolauryl, -monopalmityl, -monostearyl, -tristearyl, -monooleyl and triolcylesters; monoglycerides, e.g., glycerol mono oleate, glycerol
25 mono palmitate and glycerol monostearate, for example as known and commercially

available under the trade names Myvatex, Myvaplex and Myverol, and acetylated, e.g., mono- and di-acetylated monoglycerides, for example, as known and commercially available under the trade name Myvacet; isobutyl tallowate, n-butylstearate, n-butyl oleate, and n-propyl oleate.

- 5 (vii) Liquid silicone, for example, polyalkyl siloxanes such as polymethyl siloxane and poly(dimethyl siloxane) and polyalkyl arylsiloxane.

Other additives to the bone paste are taught in WO2005/110437 incorporated herein by reference (see for example claim 31). Examples of bone paste additive materials include polymers such as polylactones, polyamines, polymers and copolymers of trimethylene
 10 carbonate with any other monomer, vinyl polymers, acrylic acid copolymers, polyethylene glycols, polyethylenes, Polylactides; Polyglycolides; Epsilon- caprolactone; Polylactones; Polydioxanones; other Poly(alpha-hydroxy acids); Polyhydroxyalkonates; Polyhydroxybutyrates; Polyhydroxyvalerates; Polycarbonates; Polyacetals; Polyorthoesters; Polyamino acids; Polyphosphoesters; Polyesteramides; Polyfumerates; Polyanhydrides;
 15 Polycyanoacrylates; Poloxamers; Polysaccharides; Polyurethanes; Polyesters; Polyphosphazenes; Polyacetals; Polyalkanoates; Polyurethanes; Poly(lactic acid) (PLA); Poly(L-lactic acid) (PLLA); Poly (DL-lactic acid); Poly- DL-lactide-co-glycolide (PDLGA); Poly(L-lactide-co-glycolide) (PLLGA); Polycaprolactone (PCL); Poly-epsilon-caprolactone; Polycarbonates; Polyglyconates; Polyanhydrides; PLLA-co-GA; PLLA-co-GA 82:18; Poly-
 20 DL- lactic acid (PDLLA); PLLA-co-DLLA; PLLA-co-DLLA 50:50; PGA-co-TMC (Maxon B); Polyglycolic acid (PGA); Poly-p-dioxanone (PDS); PDLLA-co-GA; PDLLA-co-GA (85:15); aliphatic polyester elastomeric copolymer; epsilon- caprolactone and glycolide in a mole ratio of from about 35:65 to about 65:35; epsilon-caprolactone and glycolide in a mole ratio of from about 45:55 to about 35:65; epsilon-caprolactone and lactide selected from the
 25 group consisting of L-lactide, D-lactide and lactic acid copolymers in a mole ratio of epsilon-

caprolactone to lactide of from about 35:65 to about 65:35; Poly(L- lactide and caprolactone in a ratio of about 70:30); poly (DL-lactide and caprolactone in a ratio of about 85:15); poly(DL-lactide and caprolactone and glycolic acid in a ratio of about 80:10:10); poly(DL-lacticde and caprolactone in a ratio of about 75:25); poly(L-lactide and glycolic acid in a ratio of about 85:15); poly(L-lactide and trimethylene carbonate in a ratio of about 70:30); poly(L-lactide and glycolic acid in a ratio of about 75:25); Gelatin; Collagen; Elastin; Alginate; Chitin; Hyaluronic acid; Aliphatic polyesters; Poly(amino acids); Copoly(ether-esters); Polymethyl methacrylate (PMMA), Polyalkylene oxalates; Polyamides; Poly(iminocarbonates); Polyoxaesters; Polyamidoesters; Polyoxaesters containing amine groups; and Poly(anhydrides). The polymer can also be copolymer or terpolymer. It can be a blend of two or more individual substances mixed together. Accordingly, bone paste embodiments may include CNPs and one or more osteoinductive and/or osteoconductive components, and optionally a carrier component and/or a bone paste additive component.

Figure 1 shows a schematic of mechanisms by which osteolysis is thought to occur. Large debris (20-100 μ m) leads to “frustrated phagocytosis” which activates the NALP3 inflammasome via increases in reactive oxygen species (ROS) and reactive nitrogen species (RNS). Whereas smaller particles (<10 μ m) activate the NALP3 inflammasome by destabilizing endosome and leaking cathepsins into the cytoplasm[5]. The level of both mRNA and secreted pro-inflammatory cytokines such as IL-1, IL-6, IL-12, tumor necrosis factor (TNF) and receptor activator of nuclear factor κ B ligand (RANKL) [5-8] were reported higher in bone–prosthesis interface tissue. RANKL and macrophage colony-stimulating factor (M-CSF) are also known to involve in maturation of osteoclast progenitors into multinucleated osteoclasts which are involved in bone dissolution. It has been reported that

tissues collected at the bone–prosthesis interface had increased levels of both mRNA and secreted pro-inflammatory cytokines such as IL-1, IL-6, IL-12, TNF and RANKL.

Figure 1 illustrates these processes and the manner in which the NC coated components prevent or inhibit these processes. The following pathways were identified for
5 osteolysis: 1. *De novo* osteoclastogenesis, which occurs in response to the release of M-CSF and RANKL 2. Induction of osteoblasts apoptosis; and 3. Secretions of the matrix metalloproteinases (MMPs), collagenases and tissue-processing enzymes during the inflammatory process.

The inventors herein have developed a nanoceria coating having regenerative
10 antioxidant activity in biological microenvironment and can scavenge free radicals and suppress immune reaction/osteoclast activation which may inhibit the osteolysis, and prevent/delay implant failure. The coating which may be coated on a substrate to produce an implant. The substrate may be a prosthesis in embodiments.

The degree of catalytic activity may be varied by varying the $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratio on the
15 surface cerium of the CNPs used to manufacture the NC coating, and as a function of the coating parameters utilized in preparing the NC coating. The coating on an implant retains the catalytic activity of the CNPs.

Other embodiments include methods for assembling CNPs on the surface of Ti (and other metal alloys, such as CoCrMo) such that an NC coating is formed to retain the
20 ROS/RSN catalytic properties that convey anti-inflammatory properties. The presence of NC in the milieu of the prosthetic – bone interface enhances osseous integration. This is shown via studies of the impact of nanoceria-coated titanium on NF κ B-dependent osteoclast differentiation, activation, and bone resorption, using RANKL-stimulated human osteoclast progenitors and bone resorption as a model for study.

The NC coatings disclosed enable nanoceria-dependent collapse of ROS/RNS accumulation in response to inflammatory stimulants on substrates such as titanium. Pulse electrophoretic deposition (EPD) is used to provide a homogeneous coating on metal substrates with better catalytic activity. This coating allows better cell attachment and growth
5 than known implant surfaces.

Figure 2 shows data for the topology and catalytic property of the NC coating. NC was coated on Ti substrate using pulse EPD and EPD. Figure 2A shows pulse EPD (left) and EPD (right) using NC. Figure 2A shows surface morphology of the EPD coated Ti substrate (left- pulse EPD; right- direct current). The results show EPD resulted in a more
10 homogeneous coating compared to EPD.

Coatings and their method of preparation are disclosed. The coatings may have NC with varying ratios of surface cerium in the Ce^{3+} and Ce^{4+} oxidation state. Alternatively, the coating may have CNPs with surface cerium of only one valence state, although a mixture of oxidation states is preferable. The coatings may be used to coat any compatible substrate or
15 prosthesis. Modes disclosed involve the coating of Ti and CoCrMo substrates. As mentioned above, first nanoparticles (NP) are produced. Embodiments disclosed are (a) NC with cerium predominantly in the Ce^{3+} oxidation state (NC1) and (b) NC with cerium predominantly in the Ce^{4+} oxidation state higher (NC2). However it is understood that coatings formed from these nanoparticles (using methods below, for e.g.) can have both NC 1 and N2. Alternatively
20 coatings may be formed from either NC1 or NC2.

1. Preparation of different $\text{Ce}^{3+}/\text{Ce}^{4+}$ nanoparticles ("CNP")

Cerium is a rare-earth element with fluorite lattice structure with +3/+4 oxidation states and may interchange between the two oxidation states depending on the environment. Surface $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratio of nanoparticles of this material is also known to depend on size of

the nanoparticles. Agglomeration free and smaller size nanoparticles will also determine the quality of the coating and topology of the coating.

As mentioned above, to produce the coating using the disclosed methods, first cerium nanoparticles (CNP) are provided. In certain embodiments, CNPs of 3-5 nm size with different surface Ce^{3+}/Ce^{4+} ratios are synthesized. The CNPs are then deposited on the surface of an implant grade titanium substrate (forming a nanocerium coating also referred to herein as “NC coating”). The NC coating will no longer contain ceria in nanoparticle form. It was not previously known whether NC coatings retained the same catalytic activities that were reported for CNPs. Specific techniques for depositing the CNPs were developed and disclosed herein.

CNPs are made by several different methods, including precipitation, attrition, pyrolysis(including ultrasonic nozzle spray pyrolysis) and hydrothermal synthesis with thermal plasma or with other plasmas, such as with an RF induction plasma torch. Any of these methods, or any methods known in the art can be used for CNP production. The particles can be characterized using common laboratory techniques, including various forms of electron microscopy known in the art, atomic force microscopy (AFM), dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS), powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), matrix-assisted laser methods such as MOLDI-TOF, nuclear magnetic resonance (NMR), or any other convenient method known in the art. Nanotracking analysis (NTA) can be used to detect the particles’ Brownian motion and therefore allows sizing of nanoparticles in solution.

CNPs produced herein are preferably used in an electrolyte for pulse electrodeposition onto a substrate. Therefore, CNPs are first produced before implementation into a coating process. CNPs may be synthesized having surface cerium with different ratios of oxidation state: (a) CNPs with higher Ce^{3+} (“NC1”) (more 3+ than 4+) on the particle surface and (b)

CNPs with higher Ce^{4+} ("NC2") (more 4+ than 3+) on the particle surface. However it is understood that coatings formed from these nanoparticles (using methods below, for example) can have both NC1 and NC2. Alternatively, coatings may be formed from either NC1 or NC2. According to a specific example, NC1 and NC2 may be synthesized using wet
5 chemical method, maintaining sterile environment using high purity (99.999%) cerium nitrate hexa hydrate precursor.

Bare and surface-modified CNPs are engineered by simple wet chemical methods wherein a precursor chemical (such as cerium nitrate) is oxidized in a controlled environment to yield CNPs. The redox property and oxygen buffering capacity of CNPs are governed by
10 the surface $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratio. Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) are used to determine the size and hydrodynamic size of NPs.

Figure 3 shows (A) HRTEM images of NC1; 3-5 nm NC1; (B) the interplanar spacing of a lattice representation of fluorite structure of CNPs (selected area diffraction pattern) (C) XPS spectra showing variation in $\text{Ce}^{3+}/\text{Ce}^{4+}$ in two different CNPs (solid line NC1; sphere
15 NC2). 3-5 nm particles are preferably produced (Figure 3A) and tested for physicochemical properties prior to forming the coating.

As mentioned earlier, CNP size and morphology is very important for catalytic activity as well as the coating morphology. Size and morphology of the starting nanoparticle will be analyzed using high-resolution transmission electron microscope (HRTEM) and
20 dynamic light scattering instrument (DLS). HRTEM will provide the morphology and size of the nanoparticles. Selected area diffraction pattern (SAED) is collected to reveal the crystalline structure. Particles hydrodynamic size/agglomeration are measured using DLS in both water and ethanol (ethanol will be used for pulse EPD). Crystallinity of the nanoparticle are analyzed using selected area electron diffraction and X-Ray diffraction (XRD). Surface
25 $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratios are analyzed by Electron energy-loss spectroscopy and X-ray photoelectron

spectroscopy (XPS). XPS survey spectrum is used to determine the surface impurity of the nanoparticles, if any.

Surface properties of the nanoparticles are very important as electrophoretic mobility of the nanoparticles directly influence the deposition of the nanoparticles into an NC coating.

5 Surface charge of the nanoparticles is estimated using zeta potentiometer. Hydrogen peroxide, superoxide and nitric oxide radical scavenging activity is confirmed using known assay established by the inventors herein ²³ to test redox activity of the nanoparticles.

2. Preparation of Coatings

Hydrophilic surface of the cerium oxide supports the initial cell attachment and
10 proliferation of the human mesenchymal stem cells. Therefore, a coating may be synthesized with varying pulse EPD parameters with NC1 and NC2. If depositing the CNPs via electrodeposition (described below), the dispersion contains these CNP's, usually suspended around the portion of the substrate desired to be coated and counter electrode(s). In embodiments, the coating is synthesized with NC1 or NC2, and using varying pulse EPD
15 parameters. Because the 3+ and 4+ oxidation states exhibit different modes of catalytic activity and anti-inflammatory properties, coatings having either form or both forms exhibit various levels of catalytic activity.

In an example, two different Ti materials are coated, but it is understood that other metals or appropriate substrates may be used. In this example, a Ti- substrate (dimension: 5
20 mm × 5 mm & 2 mm × 1 mm) and Ti – Particles (<0.1µm and <1µm) are used. EPD coating has been well established in the past few years for biomedical coatings. The technique is easy to implement, low-cost, fast, and can be used to make conformal coatings.

Example. Pulse EPD to Coat Substrate to form a NC Coating / Design of the Counter Electrode

Methods for using pulse EPD to coat an implant grade Ti substrate are disclosed. EPD (not pulse) may also be employed. Advantages of pulse EPD disclosed are a more uniform deposition, as explained above with reference to Figure 2A.

One of the major challenges lies in the uniform coating of CNPs on the difficult geometries of implant and controlling the surface chemistry. It is recognized this is overcome in EPD by ensuring uniform electric field intensity in the proximity to the substrate. The novelty of EPD lies in the design, construction and placement of counter electrode (anode) in the electrolytic cell which will vary as a function Ti implant shape. As demonstrated in Figure 8, the electric field intensity in a 3 electrode setup became significantly more uniform on either sides of cathode as compared to a 2 electrode setup (uniformity is demonstrated by the larger lighter area in the 3 electrode configuration). Finite elemental modeling using COMSOL is implemented to control the counter electrode design & EPD parameters to prepare coatings with the desired coating properties.

Figure 8 demonstrates the electrode design. (A) Electric field simulation in a two v. three electrodes configuration (arrow showing NC movement) (B) Coating $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratio as a function of electrode distance and time. Additionally, the selection of process parameters in EPD allows the flexibility to control the surface chemistry of cerium oxide i.e. whether $\text{Ce}^{3+}/\text{Ce}^{4+}$ or a desired ratio of each. The data of Figure 8B shows that altering electrode distance and higher deposition time results in the altering $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratio for the deposited coating. Because the two different charged species (Ce^{3+} v. Ce^{4+}) work in different modes of anti-inflammation, the ability to create coatings having either one or both charges present helps enhance the bio-catalytic property of the coating. Moreover, the inventors have determined certain parameters may be used to obtain various $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratios, e.g, the time and distance information in Figure 8B which results in various ratios. The square data points are $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratio to deposition time; round data is $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratio to electrode distance.

As a specific example, coatings may be prepared using 5 to 10 V, 1 cm distance, for 20 seconds, 1 sec pulse having surface cerium charge ratios ranging from Ce+3/Ce+4 ratios between (2.3 – 1.2).

In EPD, coating morphology is governed by the arrangement of 2-D clusters and yields coatings with varying roughness. By varying EPD parameters (current density and time) collectively 'm (mass deposited on the electrode; equation 1 & 2, below), the size of 2-D clusters and stacking nanoparticles one can achieve an optimum catalytic activity (catalase mimetic activity expressed in K_{cat}) and coating surface roughness (Root Mean Square roughness- R_q).

The mass of the deposited NC (m) on substrate is calculated using equation 1 & 2,

$$h(t) = \frac{w_0}{V\rho} \mu_e (E - \Delta E) t \text{ ----- (1)}$$

$$m(t) = A \frac{VD_d}{i_0 \rho_d} \{ (1 + \alpha t)^{1/2} - 1 \} \text{ (1)} \quad \alpha = \frac{2K i_0^2}{V} \left(\frac{\rho_d}{D_d} \right) \text{ (2)}$$

where, i_0 , current density time $t=0$, K , the deposited mass-passed charge ratio, V , the external applied voltage, ρ_d and D_d , resistivity and density of the deposited layer. The catalytic activity may be enhanced by increasing the surface area of the coating as well as morphology/ size and stacking of 2-D clusters. A regression model is developed to form an analytical relationship of "m" as a function of " K_{cat} " and " R_q ". ($m=f(K_{cat}, R_q)$).

Using pulse EPD at various parameters, an NC coated substrate with varying physical and chemical parameters may therefore be developed. For example, the Ce3+/Ce4 ratio desired may be prepared using the time and electrode distance settings set forth in Figure 8B. Note substrates of various sizes may be employed and a similar model used to obtain the ratios, thicknesses, and properties desired.

Synthesized nanoceria NC1 and NC2 both are positively charged (data not shown), therefore a cathodic EPD is employed. (If surface charge of NC is negative/or near neutral, acid treatment is used to modify the surface charge of the NC for better coating.) Platinum may be used as an anode, but other counter-electrode materials may be used. Different NC
5 coatings (including coating thickness) are developed by varying nanoparticles concentrations (5 mM and 10 mM), pH (5 & 6), electrical field (0.5 V to 5 V) and time (pulse – 1 and 5 sec).

In addition, Ti particles may be coated. Ti-particles ($<0.1\mu\text{m}$ and $<1\mu\text{m}$) are coated with NC using pulse EPD in embodiments. The inventors herein have recognized the significance of coating particles of Ti is that Ti forms debris due to wear and tear. Debris
10 generated for Ti are less than $10\mu\text{m}$ and sometimes sub-nm., therefore $<0.1\mu\text{m}$ and $<1\mu\text{m}$ Ti particles may be coated.

For particle coatings, Ti-particles will be first dip coated on top of the Ti-electrode and then NC will be coated using pulse current. Moreover, a set of Ti-particles will also be coated using simple dip coating method and will be compared with EPD coated particles for
15 catalytic activity. NC-coated Ti-particles will be selected for Aim 2 based on catalytic activity of the particles. Ti-Particles coated with NC will be used to model debris particles partially /fully coated with NC and will be used in bone resorption model.

A range of EPD parameters are disclosed. An electrostatic model as well as theoretical calculations may be used to simulate the electric field as well as particles
20 distribution on the coating substrate which vary from the embodiments disclosed. EPD is acutely controlled by the electric field lines, which are dependent on the geometry and the placement of the electrodes. In order to realize the mass and the deposition morphology of the ceria NPs as they are being deposited on Ti implant surface to form the NC coating, the current distribution is modeled using an electrochemistry module in COMSOL software.

Figure 11(A) shows the intensity of the electric field of the electrodes obtained by finite element modeling using COMSOL. Figure 11B shows the open circuit potential (OCP) of uncoated (bare) and ceria coated samples obtained using an electrochemical test indicating a shift of potential from negative to positive. Figure 11A shows the electrical field distribution on the surface of the electrode, which is directly proportional to the amount of NC deposition. The electric field numbers in the Figure use electric field units of N/C. A 3-D geometry consisting of two sheet electrodes of dimensions 1"x1" separated by a distance of 5 mm was discretized using finite element mesh consisting of triangular elements having a highest size of 20 μm . The Ti electrode was grounded and a 60 V DC potential is applied between the electrodes. The ionic mobilities of CNPs (present in a dispersion suitable for EPD) as calculated from the zetasizer are assigned to the nanoparticles to obtain the ionic current distribution. The charge integration over the deposition time period shows the uniformity of the coating and the CNPs mass deposited. On the other hand, coating thickness will be very important for the anti-inflammatory property. According to one embodiment, a thin coating (of NC) (200-500 nm) is preferable, as probabilities of NC coated debris formation will be high rather than separate NC debris formation. Moreover, internal stress of the coating for thickness in the range of 200-500 nm will be limited to a certain extent and wear debris would be coated with the NC coating rather than uncoated debris. NC presence on the surface of the debris is preferable to, for instance uncoated Ti, because the NC coating suppresses the cascade of reactions responsible for inducing periprosthetic osteolysis.

Equation (1) is used to predict the coating thickness, where w_0 is starting weight of the solid particles in the solution, μ_e electrophoretic mobility, V , volume and ρ , suspension resistivity, E the applied direct-current voltage, and ΔE the voltage drop across the deposited layer. Electrophoretic mobility can be obtained using DLS measurement and ΔE is negligible voltage operating in this study (<100 V), therefore this equation may be used to predict the

relationship between the time and thickness for a given voltage. It has been shown that <100 V and short deposition time (180s), the thickness growth is proportional to time. Therefore, the electrodeposition process may occur over a range of time intervals to result in the thickness desired. After coating, substrates may be heated ~250 °C for 2hr for better
5 adhesion. The heating step may also be performed temperatures near 250 degrees and for a range of time. For instance, this step may also be heating the substrate to 200-450°C for 1-2.5 hr.

Direct current and pulse EPD is shown in the Figure 2A. Nanostructures are retained on both types of NC coatings. It is clear from the scanning electron microscopy that pulse
10 EPD resulted in a more even coating compared to direct current EPD. In addition to EPD or pulse EPD, dip coating may be used in certain embodiments. For example, dip coating is used for glass cover slips and Ti-particles (<0.1µm and <1µm), and electrophoretic deposition (EPD) to coat the implant grade Ti & CoCrMo. Substrates disclosed are not limited to metal substrates, but also could be glass, which may be dip coated. Coated cover slips are used for
15 confocal microscopy and catalytic measurement for ease of handling. This type of coating has application to particles coatings. It has been shown that debris generated for Ti are less than 10µm and sometimes sub-nm, therefore <0.1µm and <1µm Ti particles will be selected for coating. After dip coating (varying parameters: concentration of CNP, pH and number of dips), samples will be heated at >450°C for 2hr in argon-purged atmosphere for better
20 coating adherence. EPD parameters (electric field, pH and time will be varied to optimize the coating thickness, topology and chemistry of the coating. Similar heating steps will be followed as mentioned above. Variations include applying a voltage having a DC component and/or pulse to the substrate, having one or more counter-electrodes, or where the voltage is continuous (5 to 10 V), pulsed(1 sec pulse and 1 sec off for 20 cycles), or arbitrarily
25 increasing or decreasing with time (30 to 60 sec).

Characterization of the Coating

For ease of reference, the term “CNP” as used herein refers to cerium oxide nanoparticles in particle form. Once the CNP’s have been synthesized and deposited on a substrate, they are nanoceria (NC) coatings and no longer in particle form. The term “NC” shall refer to nanoceria generally.

The properties of these coatings have been determined. Topology and surface roughness are analyzed using both scanning electron microscopy (SEM) and atomic force microscopy (AFM). SEM provides over all surface morphology and porosity of the coating, where as surface roughness data is generated using AFM. AFM data is correlated to the cell culture and cell attachment behavior.

The topology and surface roughness may be analyzed using both scanning electron microscopy (SEM) and atomic force microscopy (AMF). Figure 4 shows the different surface characteristics of two coatings manufactured using EPD coating. Coatings subjected to deposition for a longer time (6 minutes) as compared to 3 minutes had higher roughness and less attachment to RAW cells. (RAW cells were used in this instance as model cells to model cell attachment generally). Table 2 shows surface roughness at different coating times. A roughness of ~30-35 nm EPD coating was found to result in favorable cell attachment.

Table 1: Surface roughness of the coating measured using AFM

Sample description Ceria Coated Ti	Average Roughness - EPD coating only	RMS - EPD coating only
AFM of Uncoated Ti Polished with 1000 Grit	42 ± 1 nm	54 ± 2 nm
AFM of 3 Minutes of EPD on Ti Substrate	34 ± 1 nm	47 ± 2 nm
AFM of 6 Minutes of EPD on Ti Substrate	43 ± 7 nm	63 ± 16 nm
AFM of 12 Minutes EPD Coating on Ti Substrate	63 ± 6 nm	62 ± 13 nm

Figure 9 shows that EPD coated Ti-substrate are comparable to RAW cell growth as control Ti (uncoated), however an added unexpected benefit was that the growth of *S. aureus* was reduced (85%) (Figure 9), a remarkable finding. Therefore substrates matching the surface profile of uncoated titanium metal are appropriate for embodiments.

The surface roughness of coatings is characterized using a number of other surface roughness parameters, such as Rq (Root Mean Square roughness), Rv (Maximum Profile Valley depth), Rp (Maximum Profile Peak Height), Rz (Average Maximum Height of the Profile), S (Mean Spacing of Local Peaks of the Profile, Sm (Mean Spacing of profile Irregularities, D (Peak Profile Density), or Pc (Peak Count).

An embodiment comprises a very thin coating of NC (200-500 nm) as probabilities of NC coated debris formation will be high rather than separate NC debris formation. Moreover, internal stress of the coating for thickness in the range of 200-500 nm is limited to a certain extent; and wear debris from a coated article (eg. a coated implant component) would be coated with ceramic coating. NC coating presence on the surface of the debris suppresses the cascade of reactions which induce periprosthetic osteolysis, and therefore disadvantages due to mechanical wear/debris formation are reduced. Pulse electrophoretic deposition (EPD) of the NC coating provides a homogeneous coating on the substrate with better catalytic activity due to minimum alteration of the surface Ce^{3+}/Ce^{4+} ratio of NC. It was found that a uniform coating of the substrate allows better cell attachment and growth. This was shown by coating NC on Ti substrate using pulse EPD (left) and EPD (right) using NC (Figure 2A). The pulse EPD NC coating that ensures such cell attachment is achieved using following parameters 0.5 V- 5 V, concentration of nanoparticles between 5 -10 mM and 1 second pulse for 20 – 40 second in ethanol using NC surface charge of 47 mV and average particles size of 10 nm.

The average surface roughness of the coating is at least in the range of 30-40 nm in an embodiment. The component may also be coated with one NC coating or multiple NC coatings having varying surface roughness.

A range of thicknesses have been identified which do not interfere with cell attachment or proliferation. Cell proliferation and surface roughness is shown in Figure 6, which shows the correlation with surface roughness with cell proliferation. For the test in Figure 6, macrophage cells were used as a model for any cells in order to show general cell attachment. As mentioned previously, beneficial effects of the NC coating is a result of both the inducement of cell attachment and also a reduction of inflammation, such as when cells are challenged with LPS, for example. In order to obtain coatings of the range of thickness mentioned previously, a cross-section of the coating material may be analyzed using SEM to estimate the coating thickness, interface and the coating integrity. XPS may be carried out to analyze the surface chemistry of the coating to confirm catalytic property. Note crystallinity and wettability of the coating may be analyzed using XRD and contact angle analysis to correlate the cell attachment and biocompatibility. Scratch testing may also be used to estimate the adhesion strength of the coating for biomedical application in accordance with ASTM C1624 standard. Bio-compatibility of the material will be analyzed by seeding RAW cells on the substrate with and without coating. Cell density and viability of cell will be analyzed (24, 48 and 72 hrs) using fluorescence based LIVE/DEAD® Cell Viability Assays. Attachment of the cells will be determined as discussed elsewhere to understand the cell substrate interaction

Cell attachment and proliferation of the RAW cells was found to be inversely proportional with increasing roughness of the coating, as shown in Figure 6. It should be clarified here that in this Figure, the RAW cells growth and attachment were used to model general cell attachment and not, in this part of the study, to show a particular anti-

inflammatory property. Anti-inflammatory properties were shown and described elsewhere in this disclosure. As mentioned previously, the beneficial effects of the NC coating is a result of the cell attachment generally and also its ability to reduce inflammation (inflammation for example being ROS or RNS or other reactive species in a cascade of processes earlier described). A substrate with minimum/no alteration of cell proliferation was determined based on the MTT data and used for ROS, RNS and NFkB-LUC experiment. No significant changes in ROS or RNS were observed in the presence of the coating as compared with a control substrate in the absence of stimulation with LPS or LPS PMA. A significant reduction in intracellular ROS and RNS were observed in the presence of the coating when challenged with LPS for ROS or LPS + PMA for RNS. NFkB-LUC activity was also significantly reduced in presence of coating in samples challenged with LPS.

Coating integrity: To estimate the coating thickness, a cross-section of the coating material is analyzed using SEM, interface evaluation and the coating integrity. The adhesion strength of the coating with substrate is evaluated using ASTM C1624 standard. The load may be varied linearly from zero to 40N for 10mm scratch length. In that case, loading rate may be varied from 10N/min to 100N/min based on maximum applied load. These variations are used to provide the critical load for coating failure. To access the failure mode, scanning electron microscopy (SEM) and surface profilometry will be performed along the scratch distance. The critical scratch load to determine a good coating is calculated from Equation

(2): $L_{CN} = [L_{rate} \cdot (l_N / X_{rate})] + L_{start}$ Where, L_{CN} , L_{rate} , l_N , X_{rate} and L_{start} are critical scratch load (Newton) for a defined type of damage (N = number sequence), rate of force application (N/min), distance in mm between start of the scratch track and the start point of the particular type of damage in the scratch track, rate of horizontal displacement (mm/min), and preload stylus force (Newton) at the start of the scratch test. Tangential force to normal force ratio at a specific point in the scratch test is called stylus drag coefficient (DSC) is calculated from

Equation (3): $D_{SC} = L_T/L_N$ where D_{SC} , L_T and L_N are stylus drag coefficient, tangential force in the scratch test at a given point, and normal stylus force in the scratch test at a given point. For this ceramic coating, literature reported D_{SC} values are in the range of 0.2-0.4²⁶.

Coating chemistry and compatibility

5 The surface chemistry of coatings synthesized can be altered by the medium (ethanol), electric field and heating. Cold stage XPS are carried out to analyze the surface chemistry as well as any impurity of the coating and correlated to the catalytic property. Crystallinity of the NC coating can also influence cell surface interaction. Therefore, crystallinity is analyzed using XRD and correlated with *in vitro* cell attachment and
10 proliferation. Wettability and cell growth are highly dependent on each other. First, water molecules and small protein molecules are adsorbed on the implant surface. Larger proteins later replace these small proteins during second step. This protein adsorption step is influenced by wetting behavior of implant. Therefore, wettability of the coating is analyzed using contact angle analysis to correlate the cell attachment and biocompatibility. It is
15 reported in the literature that moderate wetting (hydrophilic) shows better cell adhesion, cell growth and biocompatibility. More hydrophilic implant surfaces decrease cell adhesion. Therefore, protein moderate wetting is considered optimum for cell growth, adhesion and differentiation²⁷.

Corrosion behavior of coated Ti-plate is tested electrochemically and compared with
20 control Ti.²⁸ Application of ceramic coatings reduces the corrosion current (I_{corr}) and increases the open circuit potential (E_{oc}) to high value (see Figure 11B), thus less current is allowed to pass through the cross section. The application of cerium oxide coating for anti-corrosion has also been reported²⁹. Its anti-corrosion behavior has been tested on various metallic substrates such as stainless steel^{29, 30}, galvanized steel³¹, aluminum alloy³²,
25 magnesium alloy³³. Preliminary data of the open circuit potential (OCP) shows a positive

increase in case of NC coated Ti-substrate which indicate that NC coating prevents Ti-ion desolution in simulated body fluid (Figure 11B). The corrosion test will include measurement of polarization resistance (Tafel curve) in a simulated body fluid. Corrosion studies in simulated body fluid (SBF) are carried out to mimic the body environment. Several Mg based biodegradable alloys, Ti and bulk metallic glass have been tested in SBF for corrosion behavior^{34, 35}. Different ions present in SBF develop galvanic cells and their reactivity depends on their presence in the EMF series. Corrosion behavior of implant in SBF simulates the body environment and demonstrates the combined effect of ions present in EMF series at different positions. Electrochemical impedance spectroscopy is recorded to further verify the corrosion resistance.

An NC coated substrate with different physical and chemical parameters is developed for a range of catalytic activity and compatibility screening. For successful EPD coating, the NPs should be positively charged, thus allowing deposition on a negatively polarized coating electrode. If surface charge of NC is negative/or near neutral, acid treatment will be used to modify the surface charge of the NC for better coating. Control coating of Ti-particles with NC using EPD could pose some challenges due to low yield as only a thin monolayer of Ti-Particles could be coated at a time. Moreover, some EPD techniques can produce only one side coating and may also have some free NC. Atomic layer deposition (ALD) is an alternative technique,³⁶ which can be used for Ti particles coating. ALD coatings builds on layer-by-layer deposition thus high precision thickness control is achievable. An objective is mainly to provide a novel anti-inflammatory coating which scavenges local free radicals and suppresses the immune reaction causing osteolysis.

Catalytic Activity

The inventors have previously shown NC with higher levels of cerium in the +3 oxidation state exhibit superoxide dismutase activity³⁷ and that this reactivity correlates with

the level of cerium in the +3 oxidation state in a reversible manner³⁸. Likewise, NC with higher levels of cerium in the +4 oxidation state exhibit better catalase mimetic activity³⁹ that also is reduced when higher levels of cerium are present in the +3 oxidation state. The inventors also present data on the reactivity of NC with two ROS, nitric oxide (NO) radical
5 and the powerful oxidant peroxynitrite (ONOO⁻). Thus NC coatings have retained their catalytic properties that impart their core anti-inflammatory nature.

The reactivity of coated substrates with four primary molecules: 1) superoxide anion radical, 2.) Hydrogen peroxide, 3.) Nitric oxide radical and 4.) Peroxynitrite are determined. As mentioned previously, a difference is that the NC coating will be on a solid substrate and
10 not in nanoparticle form. For this reason, known methods are modified to accommodate this distinction.

First, superoxide dismutase activity is detected using a standard 1 mL quartz cuvette so that a small piece of the coated material (on glass substrate or titanium substrate or particles) will be placed directly in the bottom of the quartz cuvette so as to not block the
15 standard light path of the cuvette. This will be a general strategy that we will use to determine the reactivity with the aforementioned ROS and RNS. For each reactive molecule the general scheme for assay is described below:

(A) Superoxide radical anion. Superoxide is a short-lived radical that can only be detected by indirect means *in vitro*. A primary assay for determination of reactivity of the
20 coated materials is using an assay that has been described multiple times in our previous studies competition between the reaction of superoxide generated from hypoxanthine/xanthine oxidase as previously described³⁷. This assay measures the competition between the reaction of superoxide with ferricytochrome C and the material tested. This is an established assay for this reactive radical anion and has reliably correlated

with other assays for cerium oxide nanoparticles. An alternative to this assay is to carry out electron paramagnetic resonance studies using spin traps as previously described³⁸.

(B) Hydrogen peroxide. The reactivity of cerium oxide materials with hydrogen peroxide has been appropriately described as catalase mimetic activity. There are a variety of methods that are used to detect the level of peroxides, including hydrogen peroxide, via spectrophotometric means. To assess the reactivity of material coatings, use a straightforward UV-visible assay using a 1 mL quartz cuvette ³⁹. The present inventors already determined that NC that is dip coated on glass retains its catalase mimetic activity using this approach (Figure 2B). This not only shows that the assay can be used for this material, and for coated materials, but also indicates the core reactivity of cerium oxide with hydrogen peroxide is not lost during coating. An alternative to direct UV-visible assay for peroxide is to use Amplex Red dye that reacts with peroxide to give a resorufurin product that can also be detected by UV-visible spectrometry – albeit with a high extinction coefficient ³⁹.

(C) Nitric oxide. For detection of changes in nitric oxide radical in the presence of NC coated materials, the approach described previously ⁴⁰ may be used. Briefly, nitric oxide can be generated by a variety of approaches both chemical and biological. The use of so-called NONOates is most common, and most effective to generate a continuous stream of NO for reaction. S-nitroso-N-acetylpenicillamine (SNAP) is used to generate NO on a continuous basis in a standard reaction. This is followed by the conversion of ferrous hemoglobin to ferric hemoglobin by following changes in absorbance at 421 nm ⁴⁰. As an alternative, the concentration of NO in the presence and absence of coated materials in the presence of a copper-fluorescein conjugate may be tracked as previously described ⁴⁰. This conjugate reacts specifically with NO and generates a fluorescent product (excitation 503 nm, emission 530 nm) that directly correlates with NO levels. Using both of these methods confirms the reactivity of the cerium oxide coated materials

(D) Peroxynitrite. Peroxynitrite is likely the most oxidizing biological RNS, and its production during inflammation is likely the most damaging aspect of this response. However it is very reactive and its breakdown yields a large number of products that include radicals, peroxides and short-lived intermediates. In order to determine reactivity of cerium oxide coated materials, the concentration of peroxynitrite is measured as opposed to any downstream products given the difficulty in interpreting this data. The decay of peroxynitrite is measured by UV visible absorption in the presence or absence of cerium oxide coated materials ⁴¹. The present inventors have previously followed the reaction of cerium oxide nanomaterials with peroxynitrite, and found that cerium oxide accelerated the decay of peroxynitrite much in the same way that known scavengers do (uric acid for example).

To evaluate if coating retains the radical scavenging activity for quality control of the produced coatings, *in situ* degradation of H₂O₂ is analyzed. UV-Vis spectroscopy clearly indicates NC coating was able to scavenge H₂O₂ (Figure 2B). Figure 2B shows real time degradation of H₂O₂ followed absorbance at 240 nm. If substrates are coated with mixtures of NC that contain both types of particles (3+ higher SOD activity and 4+ peroxide & NO activity), the full range of catalytic activities are present.

Next, RAW 264.7 macrophage were grown on cover slips with or without NC coating and were stimulated with 5 µg/ml lipopolysaccharide (LPS) for 4hr and intracellular reactive oxygen species (ROS; mainly H₂O₂) were measured using 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFDA) described elsewhere ²¹. Cells grown on the NC coating showed significant less ROS/H₂O₂ as compared to control (Figure 2C). Figure 2C shows ROS estimated using H₂DCF fluorescence in NC coated substrate.

Similarly, the level of intracellular reactive nitrogen species (RNS) was also measured using aminophenyl fluorescein (APF) after stimulation with LPS (5 µg/ml) and Phorbol 12-

myristate 13-acetate (PMA; 1 μ g/ml) as described elsewhere²². Significant reduction of RNS was observed in NC coating as compared to the uncoated ones; Figure 2D shows RNS using APF fluorescence assay in NC coated substrate.

Anti-inflammatory properties using a macrophage cell culture model system

5 Evaluation of coated materials as anti-inflammatory use a macrophage cell culture model system in an embodiment. A large number of studies have demonstrated that cerium oxide nanoparticles exhibit catalytic properties in cell culture model systems (reviewed recently in ⁴²). *In vivo* animal studies have shown that cerium oxide nanoparticles can display anti-inflammatory properties, presumably based on the catalytic reactions with ROS and
10 RNS⁴³⁻⁴⁹. However it was neither previously known nor obvious as to whether this catalytic potential would be preserved following deposition to various base materials.

To determine the catalytic potential for each coating with pure ROS and RNS, there are two related goals 1.) To determine the ability of RAW cells to bind and adhere to NC coatings *in vitro* and 2.) To determine whether NC coatings can reduce inflammatory
15 responses generated from activated RAW macrophages *in vitro*. This stimulation triggers a classic respiratory burst of ROS and RNS allowing for the determination of the level of several oxidants using both live cell imaging and confocal microscopy. ^{21, 22, 50}. The response to M-CSF/CSF1 (33 ng/mL) and RANKL (66 ng/ml) in the same cell model is also measured as an alternative method for activation that is relevant to *in vivo* processes in osteolysis.

20 RAW 264.7 macrophage are grown on cover slips with or without NC coating and were stimulated with 5 μ g/ml lipopolysaccharide (LPS) for 4hr and intracellular reactive oxygen species (ROS; mainly H₂O₂) are measured using 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFDA) described elsewhere. Cells grown on the NC coating showed significant less ROS/H₂O₂ as compared to control (**Figure 2C**). Similarly, the level of

intracellular reactive nitrogen species (RNS) was also measured using aminophenyl fluorescein (APF) after stimulation with LPS (5 µg/ml) and Phorbol 12-myristate 13-acetate (PMA; 1 µg/ml). Significant reduction of RNS was observed in NC coating as compared to uncoated (**Figure 2D**).

5 **Binding and adhesion of RAW cells to NC coatings.**

RAW cells are seeded onto NC coatings in 6-well dishes in Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum. A seeding density of approximately 10% by area is used to study adherence and proliferation properties. Cells are cultured for 1-3 days and two sets of MTT assay are carried out. A.) to measure MTT reduction in the entire well
10 or B.) to remove the NC coating materials (with sterile forceps) and place them into an empty well with only culture medium and subsequently add MTT to measure only adherent cells. These experiments serve as a quantitative measure of RAW cell binding to the NC coating. Controls (no NC coating on base metal/glass substrate) and empty wells to differentiate background binding within the cell population are naturally employed.

15 **Anti-inflammatory activity exhibited by NC coatings on activated RAW cells.**

In parallel plate, RAW cells at 10% confluence are allowed to interact with either the NC coatings or control substrates overnight. After 24 the RAW cells are stimulated with either M-CSF/CSF1 (33 ng/mL) and RANKL (66 ng/ml), LPS (5µg/mL), or LPS (5 µg/mL) along with phorbol 12-myristate 13-acetate (PMA) (1µg/mL). Intracellular ROS is then
20 measured using Intracellular ROS using 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFDA) as previously described in our work ^{21, 50}. This probe is considered a general probe for ROS and likely correlates best with steady state hydrogen peroxide levels. To determine changes in RNS level the aminophenyl fluorescein (APF) is used that has been shown to correlate better with changes in the level of RNS ⁵⁰. In parallel, RAW264.7 cells
25 are transfected with the NFκB-LUC reporter and the impact of NC coatings on LPS,

LPS+PMA, or MCSF/CSF1+RANKL reporter activity to assess impact on NFkB signaling *in vitro* is assessed (see Figure 10).

It previously has been described and utilized various methods to detect changes in the level of reactive oxygen or nitrogen species (ROS or RNS). NC coatings that have good catalytic activity will reduce the levels of ROS and/or RNS in activated macrophages (RAW
5 cells) and NFkB signal activation. NC coatings that retain high levels of cerium in the +3 oxidation state will exhibit superoxide dismutase activity. Likewise for NC coatings that have higher levels of cerium in the +4 oxidation state at the surface a higher reactivity with peroxide (catalase mimetic activity) and NO is observed. Finally the decay of peroxynitrite
10 may be accelerated by either material coating.

Coatings with mixtures of NC (NC 1 and NC2) exhibit a full range of catalytic activities. Alternate approaches to both coating the substrate and testing the reactivity are other possible scenarios, and it has been shown that several methods can be applied in observing the catalytic reactivity with ROS and RNS ^{10, 21, 37-39, 46, 50}. For example electron
15 spin resonance spin traps (DEPMPO) may be used to determine whether these materials are directly reducing the level of superoxide radicals or hydroxyl radicals. These are not selected as primary methods since measuring kinetics using ESR is more difficult, but nonetheless we there are other options to measure ROS and RNS in the presence of these coatings.

Other embodiments include methods for assembling ceria nanoparticles on the surface
20 of Ti (and other metal alloys, such as CoCrMo) such that a NC coating is formed to retain the ROS/RNS catalytic properties that convey anti-inflammatory properties. The presence of NC in the milieu of the prosthetic – bone interface will enhance osseous integration. This is shown via studies of the impact of nanoceria-coated titanium on NFkB-dependent osteoclast differentiation, activation, and bone resorption, using RANKL-stimulated human osteoclast
25 progenitors and bone resorption as a model for study.

Impact of nanoceria-coated implant material on (a) osteoclast differentiation and activation, and (b) osteoblast-mediated matrix mineralization, using commercially available primary human osteoclast progenitors and human osteoblasts in culture as models for study.

5 The skeletal response to wear debris frequently results in an inflammatory periprosthetic osteolysis. The cell types responsible for bone resorption and loosening in this setting arise from the monocyte / macrophage lineage – primarily an inflammatory osteoclast (“OC”). The Rel domain transcription factors NFkB and NFATc1 play particularly important roles downstream of RANKL / TNFSF11 stimulation, a key member of the TNF superfamily
10 whose tone directs OC lineage allocation and activity via NFkB and NFATc1. Classical and alternative activation pathways entrain nuclear localization of NFkB, with ROS principally H₂O₂ amplifying and propagating NFkB activation downstream of LPS and TNF superfamily members in multiple cell types. Novack and colleagues recently demonstrated that activation NIK – a unique component of the alternative NFkB pathway activated by RANKL– was key
15 in development of the inflammatory OC, confirming the importance of this pathway.

 Whether NC will modulate ROS-dependent amplification of the RANKL / NIK (NFkB inducing kinase) / NFkB signaling pathway - and thereby mitigate inflammatory osteoclast differentiation and activity *in vitro* is assessed. Because bone-forming osteoblasts exhibit limited synthetic responses under states of inflammation and oxidative stress, the
20 impact of optimized nanoceria coatings on human osteoblast mineralize matrix formation and osteogenic differentiation are determined.

 Bone resorption by OCs requires secretion of cathepsin K (catK), a panoply of MMPs (MMP9, MMP14), and protons in resorption pits to degrade collagen and mobilize calcium and matrix factors including TGF-beta1. Basal osteoclast activity helps sustain the
25 healing angiogenic response and robust osteoprogenitor recruitment in part via MMP9, Wnt

ligands, and chemokines. However, excessive ROS reduces osteoblast mitochondrial membrane potential, synthetic activity, and increase RANKL production and osteoclast activation. Thus, strategies that mitigate an excessive oxidative inflammatory response by osteoblasts and osteoclasts lead to improved osseous integration, including MMP9 and
5 MMP14 (MT1-MMP) to degrade collagen and enable cell migration. Additionally, resorption pit acidification by V-ATPase is critical to both zymogen activity and calcium mobilization.

To address whether NC-coated surfaces mitigate programmed inflammatory responses, RAW264.7 murine osteoclast progenitors were plated along with primary vascular (adventitial) myofibroblasts on NC-coated titanium or glass surfaces. As shown in Figure 5,
10 left panel, RANKL induction of MMP9 and MMP14 was reduced when RAW264.7 cells were plated on NC coated glass. Furthermore, activation of NFkB-LUC by LPS was significantly reduced by 42% on NC-coated glass vs. uncoated glass or plastic ($p = 0.04$, $n = 6/\text{group}$). Similarly, when cultured on plastic or titanium TNF upregulated myofibroblast expression of multiple metalloproteinases including MMP9, MMP12, and MMP13. However,
15 NC-coated titanium surfaces reduced MMP9, MMP12, and MMP13 expression even in the presence of TNF (Figure 5, right panel).

The data indicate that nanoceria coatings do not impair the early phases of Lonza NHOst primary human osteoblasts in culture (Figure 7). Figure 7 shows primary human osteoblasts were plated onto type I collagen-coated (A) control or (B) NC-coated Ti plates &
20 cultured for 8 days. Alkaline phosphatase activity was visualized by Vector Red staining. As can be seen from comparing the images, no gross differences were observed, and no staining is observed on the uncoated sides.

Thus, the data shows that NC coatings reduce inflammatory osteoclast activation and bone resorption without impairing osteoblast synthetic functions as relevant to wear-debris
25 responses in periprosthetic osteolysis. This may be further validated using human primary

monocyte osteoclast progenitors (e.g. from Lonza (2T-110)). In this system, co-treatment with M-CSF/CSF1 (33 ng/mL) and RANKL (66 ng/ml) is required for 7-14 days to induce osteoclast differentiation of progenitors when cultured on OsteoAssay Human Bone Plates (Lonza PA-1000) or bone slices (IDS / Immunodiagnostics DT-1BON1000-96). Bone resorbing activity is quantified by measuring type I collagen telopeptide (CTX) released to the culture supernatants due to osteoclast actions on OsteoAssay Human Bone Plates. Key osteoclast differentiation markers (MMP9, CatK, TRAP, beta3 integrin, calcitonin receptor, DC-STAMP) are measured by RT-qPCR (Taqman Gene Expression Assay) using RNA extracted from cells cultured on OsteoAssay Human Bone.

In parallel, Western blot analysis, zymography, and ELISAs are used to quantify oxidative pro-MMP9 activity, MMP9, and cathepsin K as pathophysiologically important markers of osteoclast function. Similarly, osteoclast activity on bone (or dentine) surfaces creates resorption pits that can be quantified by digital image analysis following staining with toluidine blue. Actin double ring sealing zone formation – *sine qua non* for true osteoclast activity – is assessable by FITC-phalloidin staining and epifluorescence imaging on bone disks as described by Novack et al.⁸⁸

Briefly, in quadruplicate and in 96 well format, 10K (10,000) human osteoclast precursors are plated in 100 ul of OPBM Bullet Kit Basal Media containing either vehicle or 2X MCSF+RANKL onto either Lonza Human Bone Plates or IDS bone slice plates previously coated with 100 microliters of (a) control media (no microspheres); (b) media with <0.1 μm and <1 μm Titanium microspheres (American Elements); or (c) media with NC coated Ti-microspheres. \

The <0.1 μm and <1 μm micron particle size is initially chosen since the mean size of particulate wear debris reported in failed knee and hip arthroplasty has been reported to be between 0.5 μm and 1 μm and is engaged by phagocytosis. The smaller 0.1 μm size is

included since debris of this diameter is taken up by cells via fluid-phase pinocytosis. A dose-ranging of microsphere administration will initially encompass 1E5, 1E6, and 1E7 particles per well, for 10:1, 100:1, and 1000:1 ratios of particles per osteoclast progenitors plated. Additionally, as a positive control, co-treatment with 5 uM cardamonin - a previously
5 validated pharmacological inhibitor of RANKL/NFkb signaling in the myeloid lineage- in OPBM Bullet Kit Basal Media +/- MCSF+RANKL will be used to establish the dynamic inhibition range possible via the NFkB-dependent program regulated by NC.

Following 7 days in culture, the supernatant is analyzed for human CTX-I by CrossLaps Elisa (For culture; IDS catalog number AC-07F1) and RNA extracted from
10 monolayers (Qiagen RNeasy PLUS 96 Kit cat#74192) for analysis of human MMP9, MMP12, MMP14, Cathepsin K (major collagenase), Atp6v1c1 (osteoclast V-ATPASE proton pump, acidifies resorption pits), calcitonin receptor, beta3 integrin, DC-STAMP, and mRNAs normalized to 18S rRNA control.

Key proteins markers are evaluated as above. In parallel, cell plated as above onto
15 bone slices are cultured for a total of 14 days. As before, culture supernatants are collected for CTX-I quantification by ELISA. Following washing with PBS and ultrasonication in 250 uL of 70% isopropanol, resorption pits are visualized by toluidine blue staining (100 µl of 1% TB for 3 minutes), rinsed in ddH₂O and digital images under light microscopy of the dark blue pits quantified by ImageJ software for both area and number. In a parallel, cells plated
20 onto bone slices (vide supra) are cultured for a total of 7 days, supernatants harvested for CTX-I ELISA, and bone slice-associated osteoclasts fixed and stained with FITC-phalloidin, DAPI, and Alexa594- conjugated anti-p100/p52 (Cell Signaling RabMab clone 18D10) to image and quantify actin ring sealing zones, nuclei, and nuclear NFkB2 localization, respectively, following epifluorescent illumination.

Quantitative assessment of normal human osteoblast differentiation is also characterized as in rodent systems, assessing alkaline phosphatase activity, calcium deposition by alizarin red staining/solubilization/spectrophotometry, and osteogenic gene expression and RANKL by RT-qPCR. Osteoblast mitochondrial DNA damage will be
5 quantified by multiplex Taqman assay quantifying % human mtDNA deletion and cellular respiration / mitochondrial function assess by trypsinizing and replating nanoceria-exposed osteoblast into collagen-coated XFp Seahorse Miniplate followed by Cell Mito Stress Testing using an XFp Flux Analyzer.

No deleterious actions of NC exposure have been observed with respect to osteoblast
10 differentiation, proliferation or metabolic function –mineralization may in fact be increased due to reductions in accumulating ROS. Like cardamonin, Ti-microsphere coated with NC exhibits reduced RANKL-dependent osteoclast activity, reflected in reduced CTX-I release into culture media, reduced expression of NFkB-dependent transcripts, osteoclast pit resorption area, & decreased actin sealing zone area & nuclear NFkB2 accumulation. If pit
15 number is not reduced, this indicates selective action on resorption and migration in setting of reduced resorption area. If reduced, it may indicate either decreased osteoclastogenesis and/or reduced osteoclast progenitor viability with loss of RANKL/NFkB signaling tone. Validated human cellular reagents are used and examples are provided to encompass differentiated primary murine bone mononuclear cells in addition to murine RAW264.7 cells to initiate
20 implant studies in rodents since osseous integration of NC-coated material is examined in preclinical orthopedic models of healing and strength. Moreover, reporter mice expressing luciferase down-stream of NFkB and NFATc response elements exist that can help guide and “fine-tune” *in vivo* the transcriptional inflammatory response in addition to the specific types of cellular inflammatory foreign body responses elicited in bone to wear debris and as
25 modified by NC coatings.

The impact of nanoceria-coated titanium on NFkB-dependent osteoclast differentiation, activation, and bone resorption, is assessed using RANKL-stimulated human osteoclast progenitors and bone as a model for study. The presence of optimized NC coating onto implant surfaces reduces the pro-inflammatory ROS/RNS signaling that promotes bone-
5 resorbing osteoclast function – a primary cellular mediator of prosthetic implant loosening and impair osseous integration in ARMD osteolysis¹¹ driving TJR failure. As outlined, the skeletal response to wear debris frequently results in an inflammatory periprosthetic osteolysis⁵¹. The cell types responsible for bone resorption and loosening in this setting arise from the monocyte / macrophage lineage – primarily an inflammatory osteoclast (OC) – but
10 mesenchymal cells (wound myofibroblasts, osteoblasts) also elaborate proteases and matrix constituents that impact integration (e.g., Figure 5)⁵¹. Figure 5: MMP9, MMP12 and MMP14 expression in ceria coated substrate after induction with RANKL OR TNF.

The Rel domain transcription factors NFkB and NFATc1 play particularly important roles downstream of RANKL / TNFSF11 stimulation, a key member of the TNF superfamily
15 whose tone directs OC lineage allocation and activity via NFkB and NFATc1¹¹⁻¹³. Classical and alternative activation pathways entrain nuclear localization of NFkB, with ROS principally H₂O₂ amplifying and propagating NFkB activation downstream of LPS and TNF superfamily members in multiple cell types⁵²⁻⁵⁴. Novack and colleagues recently demonstrated that activation NIK – a uniquely component of the alternative NFkB pathway
20 activated by RANKL– was key in the development of the inflammatory OC, confirming the importance of this pathway¹¹. Bone resorption by OCs requires expression and secretion of cathepsin K (catK) and panoply of MMPs including MMP9 and MMP14 (MT1-MMP) to degrade collagen and enable cell migration. Additionally, resorption pit acidification by V-ATPase is critical to both zymogen activity and calcium mobilization. Since human bone
25 cells (osteoclasts, mesenchymal osteoprogenitors) represent the clinically relevant cell type

whose behavior is the focus of the disclosed embodiments, the tests for the impact of NC-coated surfaces upon basal and RANKL-stimulated human osteoclast bone resorption is performed using the OsteoAssay Human Bone Plate from Lonza⁵⁵.

Human primary monocyte osteoclast progenitors may be purchased from Lonza (2T-110). In this system, co-treatment with M-CSF/CSF1 (33 ng/mL) and RANKL (66 ng/ml) is required for 7-14 days to induce osteoclast differentiation of progenitors when cultured on OsteoAssay Human Bone Plates (Lonza PA-1000)⁵⁵ or bone/dentine slices⁵⁶ (IDS / Immunodiagnostics DT-1BON1000-96). Bone resorbing activity can be quantified by measuring type I collagen telopeptide (CTX) released to the culture supernatants due to osteoclast actions on OsteoAssay Human Bone Plates. Key osteoclast differentiation markers (MMP9, CatK, TRAP, beta3 integrin, calcitonin receptor, DC-STAMP) are measured by RT-qPCR (Taqman Gene Expression Assay) using RNA extracted from cells cultured on OsteoAssay Human Bone using the methods we've previously detailed⁵⁷⁻⁵⁹. Similarly, osteoclast activity on bone (or dentine) surfaces creates resorption pits that can be quantified by digital image analysis following staining with toluidine blue on IDS bone slices as described. Actin double ring sealing zone formation – *sine qua non* for true osteoclast activity – is assessable by FITC-phalloidin staining and epifluorescence imaging on bone disks as described by Novack et al.^{12, 13} Briefly, in quadruplicate and in 96 well format, 10K (10,000) human osteoclast precursors will be plated in 100 ul of OPBM Bullet Kit Basal Media containing either vehicle or 2X MCSF+RANKL onto either Lonza Human Bone Plates or IDS bone slice plates previously coated with 100 microliters of (a) control media (no microspheres); (b) media with <0.1 µm Titanium microspheres; (c) media with <1 µm Titanium microspheres; (d) media with NC coated <0.1 µm Ti-microspheres; or (e) media with NC coated < 1 µm Ti-microspheres. The <0.1µm and <1 µm micron particle sizes are initially chosen since the mean size of particulate wear debris reported in failed knee and hip

arthroplasty has been reported to be between 0.5 μm and 1 μm and is engaged by phagocytosis^{60, 61}. The smaller 0.1 μm size is included since debris of this diameter is taken up by cells via fluid-phase pinocytosis⁶². A dose-ranging of microsphere administration will initially encompass 1E5, 1E6, and 1E7 particles per well, for 10:1, 100:1, and 1000:1 ratios of particles per osteoclast progenitors plated. Additionally, as a positive control, co-treatment with 5 μM cardamonin -- a previously validated pharmacological inhibitor of RANKL/NF κ b signaling in the myeloid lineage⁶³-- in OPBM Bullet Kit Basal Media +/- MCSF+RANKL will be used to establish the dynamic inhibition range possible via the NF κ B-dependent program regulated by NC. Following 7 days in culture, the supernatant will be analyzed for human CTX-I by CrossLaps Elisa for bone resorption (For culture; IDS catalog number AC-07F1) and RNA extracted from monolayers (Qiagen RNeasy PLUS 96 Kit cat#74192) for RT-qPCR analysis of human osteoclast markers MMP9, MMP12, MMP14, Cathepsin K (major collagenase), Atp6v1c1 (osteoclast V-ATPASE proton pump, acidifies resorption pits), calcitonin receptor, beta3 integrin, DC-STAMP, and mRNAs normalized to 18S rRNA control. In parallel, cell plated as above onto bone slices will be cultured for a total of 14 days. As before, culture supernatants are collected for CTX-I quantitation by ELISA^{64, 65}. Following washing with PBS and ultra sonication in 250 μL of 70% isopropanol, resorption pits are visualized by toluidine blue staining (100 μL of 1% TB for 3 minutes), rinsed in ddH₂O and digital images under light microscopy of the dark blue resorption pits quantified by digital image analysis with ImageJ software⁶⁶ for both area and number¹¹⁻¹³. In a parallel, using techniques previously implemented^{57, 67}, cells plated onto bone slices (vide supra) are cultured for a total of 7 days, supernatants harvested for CTX-I ELISA, and bone slice-associated osteoclasts fixed and stained with FITC-phalloidin, DAPI, and Alexa594-conjugated anti-p100/p52 (Cell Signaling RabMab clone 18D10) to image and quantify actin ring sealing zones, nuclei, and nuclear NF κ B2 localization, respectively, following

epifluorescent illumination (nuclear NFkB2:RelB complex key to RANKL osteoclast differentiation;^{12, 13}.

In parallel, normal human osteoblasts (Clonetics NHost; Lonza #CC-2538) are expanded then cultured in 12 well cluster plates (50,000 cell/cm²) on mineralization media (10% FBS, alphaMEM supplemented with 3 mM beta-glycerol phosphate + 50 ug/ml ascorbic acid) in (a) the presence or absence of 5 ug/ml lipopolysaccharide (LPS) and (b) NC-coated vs. uncoated titanium microspheres at 0, 4E6, 4E7, and 4E8 spheres per well (corresponding to ca. 0, 10:1, 100:1, and 1000:1 spheres per osteoblast at confluence). All experimental sets are performed in quadruplicate, encompassing both <0.1 micron and <1 micron spheres. Cells are re-fed twice per week for 21 days, with fresh media and spheres added with each feeding following gentle rinsing in warmed media. At the end of the culture period, cultures are processed for quantification of calcification by Alizaran red staining we've previously detailed^{57, 58, 67}. Conditioned media is collected at each refeeding and processed weekly for type I collagen propeptide measurement by ELISA (P1NP; bone formation and matrix synthesis marker¹⁶). Elaboration of the osteogenic gene regulatory program is quantified by RT-qPCR (initially querying bone alkaline phosphatase = alp2/TNAP, bone sialoprotein, osteocalcin, Col1A1 and Col1A2 vs. Col2A1 and Col10A1, Runx2, Osx, Msx2, RANKL, OPG, MMP9, MMP12, MMP14/MT1MMP, TNF,) with normalization to 18S rRNA. In separate 24 well-culture format, human osteoblast / NHOST apoptosis will be assays using the Cayman Multiparameter Apoptosis Fluorescence Assay (#600330; 96 well capable but 24 well preferred re: plating), scoring LPS-induced apoptotic responses in the presence or absence of the NC-modified Ti microspheres dose-response. LPS treatment is chosen since this a (patho) physiologically relevant stimulus that conveys risk for failure of prosthetic osseous integration *in vivo*. Should significant differences exist between titanium and NC-coated titanium cultures with respect to osteogenic mineralization

and/or P1NP / type I collagen synthesis, an exploratory analysis may be undertaken with UTSW Genomice and Microarray Facility implementing PrimeView Human Gene Expression Array (best reproducible coverage of the ANNOTATED human transcriptome) with RNA samples possessing RIN $\geq$ 7 as done in our analyses of murine mineralizing tissues^{57, 58}.

Based upon the data presented in the Figures herein, the Ti-microsphere coated with NC will exhibit reduced RANKL-dependent human osteoclast activity, reflected in reduced CTX-I release into culture media, reduced expression of NFkB-dependent transcripts (e.g., MMP9 and MMP14, potentially CatK, others), reduced osteoclast pit resorption area, & decreased actin sealing zone area & nuclear NFkB2 accumulation. Pit number may or may not be reduced. If pit number is not reduced, this indicates selective action on resorption and migration in setting of reduced resorption area. If reduced, it may indicate either decreased osteoclastogenesis and/or reduced osteoclast progenitor viability with loss of RANKL/NFkB signaling tone.

Furthermore, it follows that (a) human osteoblast (NHOST) mineralization will not be inhibited by exposure to NC-coated Ti-microsphere; and (b) osteogenic differentiation and mineralization are restored by NC-coated Ti microspheres in LPS-treated NHOST cultures. As in osteoclasts, targets of LPS-elicited NFkB signaling in osteoblasts (e.g., the above MMPs, TNF expression itself) and NHOST apoptosis are reduced by exposure to NC-coated Ti microspheres. Because titanium itself has been variably described as enhancing osteoblast synthetic function⁶⁸, it may be that NC-coating might diminish basal osteoblast activity but restore LPS-inhibited mineralization via its “catalase-like” activity. In this case, optimization of NC-coating mass / titanium surface area is iteratively pursued using methods described above and metrics of osteoblast function, to retain anti-inflammatory function while minimizing any potential basal advantage of titanium implant exposure.

Example: *In vivo* use of coated substrates

To assess how nanoceria coating of titanium impacts inflammation, net bone mass accrual, and skeletal tissue repair *in vivo*, diabetic mice are used as a model for study. Patients with type II diabetes (T2D) are particularly prone to lower extremity arthritis, fracture, and amputation in part due to the low-grade systemic inflammatory state of the disease, with concomitant neuropathy and vasculopathy^{2 4-7}. Preclinical models of dysmetabolic disorders such as diabetes, dyslipidemia, and chronic kidney disease recapitulate the pro-inflammatory states that impair fractures healing and osseous integration. Therefore, the model is implemented in a well-defined murine model of T2D and dyslipidemia – the male low density lipoprotein receptor null mouse (LDLR^{-/-}) fed high fat diet¹⁴⁻¹⁹ – to characterize the impact of nanoceria coatings on bone histomorphometric / histological response to titanium implant. Biomechanical strength and osseointegration of NC-coated Ti vs. uncoated Ti are thereby confirmed *in vivo* as well as supporting *in vitro* data.

Because inflammatory ROS/RNS signaling upregulates bone-resorbing osteoclast differentiation and monocyte/macrophage activities while suppressing osteoblast-mediated bone formation, the ROS/RNS catabolic activities of NC-coated titanium implants enhance healing and osseous integration around Ti prosthetics *in vivo*. Because the dysmetabolic state of T2D and metabolic syndrome impairs skeletal repair processes *in vivo* in both clinically relevant murine disease models^{14, 69} and in human patients⁴⁻⁷, the NC coating is evaluated on osseous integration in the LDLR^{-/-} mouse fed high fat diabetogenic diet^{18, 57}. Since distal femurs implant model with “push in” mechanical testing has been recently established to evaluate osseointegration in the setting of murine chronic kidney disease (CKD)^{20, 70}, the present inventors deploy this novel mechanical testing system that has emerged as more

sensitive than pull out in preclinical assessments of osseous integration⁷¹. All animal data collection and presentation are in compliance with ARRIVE guidelines^{72,16, 57, 67,73}.

To register NFkB signaling *in vivo*, NFkB-LUC reporter mice are utilized, B10.Cg-*H2^k* Tg(NFkB/Fos-luc)26Rinc/J (stock #006100) using firefly luciferase
 5 IMMUNOHISTOCHEMISTRY⁷⁴ alongside nuclear NFkB2 histochemistry. Although in culture there is a nanoceria surface- dependent reduction in NFkB-driven transcriptional activity by luciferase assay following LPS stimulation (Figure 10), the expression of skeletal tissue expression must encompass immunohistochemistry using the methods previously demonstrated^{57, 58, 67}. To enable this, NFkB-LUC reporter mice are bred for 5 generations
 10 onto the LDLR^{-/-} parental C57BL/6J background to enable diet-induced diabetes and dyslipidemia as others and we have previously described^{18, 57, 75}. The line will be expanded and 100 male NFkB-LUC;LDLR^{-/-} male siblings challenged with high fat diet (Paigen formulation of the diabetogenic High Fat Diet^{14, 18}) to induce diabetes, dyslipidemia, and bone disease (Figure 12). After 4 weeks, animals will be fasted for 4 hours and retro orbital
 15 blood obtained under tribromomethanol anesthesia to confirm increased fasting blood glucose, insulin, and HOMA-IR as we've previously shown⁵⁷. Once confirmed, the two days later the hair of the right lower extremity is shaved under anesthesia and using aseptic techniques, a unilateral distal right femoral lateral-to-medial submetaphyseal hole will be introduced orthogonal to the lateral aspect by sequential 0.7 mm and then 1.0 mm with a
 20 Dremmel hand drill and sterilized stainless steel bits -- with normal saline irrigation for tissue cooling^{20, 70}. A sterile 1 mm by 2 mm implant (NC-coated titanium vs. titanium, sterilized in 100% ethanol and dried, no UV sterilization to avoid potential confounding photofunctionalization) is placed in the femur by gentle tamping, and wound closed with 6-0 silk to secure overlying muscle and fascia, and 5-0 silk for skin closure. Two x 0.4 cc normal
 25 saline (35C warmed) subcutaneous fluid "boluses" administered (one over each flank) per

mouse (one just before, one just after surgery) along with 0.05 mpk of subQ buprenorphine for pain control, and the mouse allowed to recover from surgery and anesthesia on a warming blanket with close post-operative monitoring.

The following day, high fat diet continues to be provided but now within the cage
5 during recovery with water gel pack hydration and daily buprenorphine 0.05 mpk for three days. Our own experience with invasive surgery femoral surgery to date yields a ca. 10% perioperative mortality. Being more conservative, 15%=16% dropout rate is assumed, enabling at least 41 mice in each group to continue to experience HFD –induced diabetes, dyslipidemia, and bone disease for an additional 4 weeks. At the end of the dietary challenge,
10 mice are fasted 4 hours with ad lib access to water then sacrificed by exsanguination under general anesthesia for harvest and analysis of femoral tissues as outlined below. Our personal histological assessment of bone and other mineralized tissues place the need for histology / histomorphometry at a minimum of 5 animals per group per replicate^{58, 76-78}. Any additional animals will be tasked to histomorphometry given its variance^{58, 76-78}. For push-in
15 mechanical loading, 10 mice are included per arm based upon the recent published literature of Lanske and colleagues^{20, 70}.

Histomorphological assessment of bone implant contact interface and adjacent bone volume to tissue volume in non-decalcified plastic sections (n = 5 per group). Non-decalcified bone plastic sections will be prepared essentially as we've previously described^{58, 77, 78}, but using a saw microtome as below. The distal 1/3 of the implanted femur is harvested,
20 carefully cleared of adherent tissue and fixed en bloc overnight in 70% ethanol, dehydrated in graded acetone at 4C (70% x 2hrs, 90% x 1 hours, 100% x 2 hours).

Samples are sequentially infiltrated with 85% methylmethacrylate/15% dibutylphthalate/0.15% benzoyl peroxide in a 20 ml glass Wheaton vial a vacuum desiccator
25 for 48 hours at 4C). Methacrylate polymerization⁷⁹ solution is then initiated at 37C in a

vacuum oven with fresh 85% methymecrylate/15% dibutylphthalate/but now with 5% benzoyl peroxide for 48hours (>20 vol per tissue vol). Following cooling to -20C for 30 minutes, the glass vial is carefully broken with a hammer and the plastic block containing distal femurs with implant manually trimmed with a band saw. Subsequent sectioning (50
5 micron) with a Leica SP1600 diamond saw microtome (cuts soft metals including titanium as well as bone), cutting sections longitudinal / parallel to the long axis of the implant onto chrom-alum gel coated slides. After deplastination in Cellsolve and rehydration through graded ethanol, bone tissue including osteoblasts and osteoids is visualized by 1% toluidine blue (pH = 3.7), rinsed in citrate buffer, dehydrated in butanol/xylenes, and mounted
10 (Permount) and cover slipped. Digital JPEG photomicrographs of the terminal implant bone interface (3 per specimen) are analyzed by ImageJ64 / BoneJ plugin in lieu of osteomeasure^{58, 77, 78} to characterize the distal 5% / 0.1 mm of implant – encompassing approximately 2 x 0.1 mm + 1 mm cylinder diameter = 1.2 mm of implant surface – quantifying the bone-implant contact surface (percent BS/IS = bone surface to implant surface) and bone volume to tissue
15 volume (BV/TV)⁸⁰ in the 250 micron segment abutting the distal end of the implant.

Example: Immunohistological assessment of NFkB activation and osteoclast numbers in bone adjacent to femoral implants in decalcified paraffin sections (n = 5 / group).: The distal 1/3 of the implant femur is harvested, carefully cleared of adherent tissue and fixed for 2 days in 10% neutral buffer formalin with shaking at 4C in 20 ml glass Wheaton vials.
20 Subsequently, tissue is decalcified for 3 week using 0.375M EDTA pH8 at 4 with stirring⁵⁸. Samples are then embedded in paraffin by the Pathology Core, and 5 micron paraffin sections cut *orthogonal* to the long axis of the implant, prepared from medial to lateral surface of the distal femur until the implant is encountered.

Luciferase reporter expression, nuclear NFkB accumulation, TRAP and TNAP (bone
25 alkaline phosphatase^{58, 81}) elaboration is assessed by immunohistochemistry in adjacent

sections at 15-40 micron (Zone 1; roughly 1 osteoclast diameter), 70-90 micron (Zone 2), 140-160 micron (Zone 3), and 200-220 micron (Zone 4 – approaching contralateral subperiosteum) penumbra distances from the distal end of the implant using the Vector ImmPress immunohistochemical dendrimer HRP enhanced method previously
 5 implemented^{57, 58, 81} with light hematoxylin counterstaining. (Note: the 10 mM H2O2 used to quench endogenous peroxidase far exceeds the catalytic activity of nanoceria, and any background signal is identified with control sections lacking incubation with primary or secondary antibodies).

Digital photomicrographs are analyzed by ImageJ64 / BoneJ plugin to characterize
 10 the expression area (% of tissue volume) of the luciferase NFkB reporter by immunohistochemistry, TRAP (osteoclast marker), TNAP (osteoblast marker), and nuclear NFkB2 (inflammatory NFkB activation marker) in the 4 zones abutting the inserted end of the titanium implants with or without nanoceria coatings.

**Example: Assessment of integration using push-in uniaxial load testing of
 15 titanium implants with or without nanoceria coating:**

Uniaxial “push in” loading following the method of Lanske^{20, 70} is implemented to characterize osseous integration, a technique adopted from the dental literature. The push-in test has emerged as more sensitive than the pull-out test for mechanical assessment of the bone-implant interface in preclinical models⁷¹. Briefly, the entire right femur containing the
 20 distal implant will be harvested, carefully cleared of adherent tissue. The entire bone to the level of the implant site (side up) is placed in a rectangular cassette preloaded with self-polymerizing acrylic (Jet Denture Repair Acrylic), then anchored to the platen of an Instron 5944 universal microtester. The compression “needle” chuck (Instron S1-11862-1) is fitted with a 2 cm long x 0.8 mm diameter stainless steel loading pin (Washington University
 25 School of Medicine Instrument Machine Shop; Dennis Tapella, Mgr), and uniaxial

compression loading of the bone-integrated titanium prosthetic delivered 1 mm/minute along the long axis^{20, 70}, collecting force-displacement data with the Bluehill 3 Testing Software for Windows 7 and accompanying Biomedical Materials and Devices Module. Force-displacement curves will be analyzed characterizing, yield point, ultimate stress, and area under curve to characterize resistance of periprosthetic underlying bone to fracture^{20, 70, 82}. Additionally, force-displacement curves to identify regions optimally characterizing the tissue modulus are analyzed.

The NC-coated titanium implants in the *in vivo* model may exhibit increased bone – implant contact surface, reduced NFkB activation and osteoclast formation (TRAP immunohistochemistry), improved osteoblast numbers / bone formation activity at the interface (TNAP immunohistochemistry), and increased adjacent bone volume to tissue volume (BV/TV)⁸⁰ as compared with titanium implants alone. Push-in testing may reveal improved mechanical strength at the implant / bone interface in the presence of NC coating.

A dietary challenge is introduced in this procedure to induce the diabetic and dyslipidemia state in all LDLR^{-/-} mice; this could represent too severe an impairment of bone healing¹⁴ for nanoceria or NP's to overcome alone. In that event, the impact of coatings on intermittent PTH-induced bone formation -- a strategy that has been shown to reduce oxidative stress in bone^{84, 85} and vasculature¹⁶ and recently demonstrated to enhance osseous integration in mice⁸⁶ – are examined using the general outline in Figure 12.

The above description is provided as an aid in examining particular aspects of the invention, and represents only certain embodiments and explanations of embodiments. The examples are in no way meant to be limiting of the invention scope. The materials and methods provided below are those which were used in performing the examples that follow.

It should be borne in mind that all patents, patent applications, patent publications, technical publications, scientific publications, and other references referenced herein are

hereby incorporated by reference in this application in order to more fully describe the state of the art to which the present invention pertains.

Reference to particular buffers, media, reagents, cells, culture conditions and the like, or to some subclass of same, is not intended to be limiting, but should be read to include all such related materials that one of ordinary skill in the art would recognize as being of interest or value in the particular context in which that discussion is presented. For example, it is often possible to substitute one buffer system or culture medium for another, such that a different but known way is used to achieve the same goals as those to which the use of a suggested method, material or composition is directed.

It is important to an understanding of the present invention to note that all technical and scientific terms used herein, unless defined herein, are intended to have the same meaning as commonly understood by one of ordinary skill in the art. The techniques employed herein are also those that are known to one of ordinary skill in the art, unless stated otherwise. For purposes of more clearly facilitating an understanding the invention as disclosed and claimed herein, the following definitions are provided.

While a number of embodiments of the present invention have been shown and described herein in the present context, such embodiments are provided by way of example only, and not of limitation. Numerous variations, changes and substitutions will occur to those of skill in the art without materially departing from the invention herein. For example, the present invention need not be limited to best mode disclosed herein, since other applications can equally benefit from the teachings of the present invention. Also, in the claims, means-plus-function and step-plus-function clauses are intended to cover the structures and acts, respectively, described herein as performing the recited function and not only structural equivalents or act equivalents, but also equivalent structures or equivalent acts, respectively. Accordingly, all such modifications are intended to be included within the

scope of this invention as defined in the following claims, in accordance with relevant law as to their interpretation.

References

1. Murphy LB, Helmick CG, Allen KD, Theis KA, Baker NA, Murray GR, Qin J, Hootman JM, Brady TJ, Barbour KE, Centers for Disease C, Prevention. Arthritis among veterans - united states, 2011-2013. *MMWR. Morbidity and mortality weekly report*. 2014;63:999-1003
2. King KB, Findley TW, Williams AE, Bucknell AL. Veterans with diabetes receive arthroplasty more frequently and at a younger age. *Clinical orthopaedics and related research*. 2013;471:3049-3054
3. Beck RT, Illingworth KD, Saleh KJ. Review of periprosthetic osteolysis in total joint arthroplasty: An emphasis on host factors and future directions. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society*. 2012;30:541-546
4. Wukich DK. Diabetes and its negative impact on outcomes in orthopaedic surgery. *World J Orthop*. 2015;6:331-339
5. Watts CD, Houdek MT, Wagner ER, Abdel MP, Taunton MJ. Insulin dependence increases the risk of failure after total knee arthroplasty in morbidly obese patients. *The Journal of arthroplasty*. 2015
6. Rodriguez EK, Boulton C, Weaver MJ, Herder LM, Morgan JH, Chacko AT, Appleton PT, Zurakowski D, Vrahas MS. Predictive factors of distal femoral fracture nonunion after lateral locked plating: A retrospective multicenter case-control study of 283 fractures. *Injury*. 2014;45:554-559

7. Yang Z, Liu H, Xie X, Tan Z, Qin T, Kang P. The influence of diabetes mellitus on the post-operative outcome of elective primary total knee replacement: A systematic review and meta-analysis. *Bone Joint J.* 2014;96-B:1637-1643
8. Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the united states from 2005 to 2030. *The Journal of bone and joint surgery. American volume.* 2007;89:780-785
9. Purdue PE, Koulouvaris P, Nestor BJ, Sculco TP. The central role of wear debris in periprosthetic osteolysis. *HSS journal : the musculoskeletal journal of Hospital for Special Surgery.* 2006;2:102-113
10. Das S, Dowding JM, Klump KE, McGinnis JF, Self W, Seal S. Cerium oxide nanoparticles: Applications and prospects in nanomedicine. *Nanomedicine.* 2013;8:1483-1508
11. Yang C, McCoy K, Davis JL, Schmidt-Supprian M, Sasaki Y, Faccio R, Novack DV. Nf- κ B inhibition in osteoclasts results in osteoporosis and enhanced inflammatory osteolysis. *PloS one.* 2010;5:e15383
12. Vaira S, Johnson T, Hirbe AC, Alhawagri M, Anwisye I, Sammut B, O'Neal J, Zou W, Weilbaecher KN, Faccio R, Novack DV. Relb is the nf- κ B subunit downstream of nf- κ B responsible for osteoclast differentiation. *Proceedings of the National Academy of Sciences of the United States of America.* 2008;105:3897-3902
13. Vaira S, Alhawagri M, Anwisye I, Kitaura H, Faccio R, Novack DV. Relb/p65 promotes osteoclast differentiation by blocking a rankl-induced apoptotic jnk pathway in mice. *The Journal of clinical investigation.* 2008;118:2088-2097
14. Pirih F, Lu J, Ye F, Bezouglaia O, Atti E, Ascenzi MG, Tetradis S, Demer L, Aghaloo T, Tintut Y. Adverse effects of hyperlipidemia on bone regeneration and strength.

Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2012;27:309-318

15. Thompson B, Towler DA. Arterial calcification and bone physiology: Role of the bone-vascular axis. *Nat Rev Endocrinol.* 2012;8:529-543
- 5 16. Cheng SL, Shao JS, Halstead LR, Distelhorst K, Sierra O, Towler DA. Activation of vascular smooth muscle parathyroid hormone receptor inhibits wnt/beta-catenin signaling and aortic fibrosis in diabetic arteriosclerosis. *Circulation research.* 2010;107:271-282
17. Lai CF, Seshadri V, Huang K, Shao JS, Cai J, Vattikuti R, Schumacher A, Loewy AP,
10 Denhardt DT, Rittling SR, Towler DA. An osteopontin-nadph oxidase signaling cascade promotes pro-matrix metalloproteinase 9 activation in aortic mesenchymal cells. *Circulation research.* 2006;98:1479-1489
18. Towler DA, Bidder M, Latifi T, Coleman T, Semenkovich CF. Diet-induced diabetes activates an osteogenic gene regulatory program in the aortas of low density
15 lipoprotein receptor-deficient mice. *The Journal of biological chemistry.* 1998;273:30427-30434
19. Shao JS, Sierra OL, Cohen R, Mecham RP, Kovacs A, Wang J, Distelhorst K, Behrmann A, Halstead LR, Towler DA. Vascular calcification and aortic fibrosis: A bifunctional role for osteopontin in diabetic arteriosclerosis. *Arterioscler Thromb
20 Vasc Biol.* 2011;31:1821-1833
20. Sun N, Guo Y, Liu W, Densmore M, Shalhoub V, Erben RG, Ye L, Lanske B, Yuan Q. Fgf23 neutralization improves bone quality and osseointegration of titanium implants in chronic kidney disease mice. *Scientific reports.* 2015;5:8304
21. Das S, Singh S, Dowding JM, Oommen S, Kumar A, Sayle TX, Saraf S, Patra CR,
25 Vlahakis NE, Sayle DC, Self WT, Seal S. The induction of angiogenesis by cerium

oxide nanoparticles through the modulation of oxygen in intracellular environments.

Biomaterials. 2012;33:7746-7755

22. Dickinson BC, Huynh C, Chang CJ. A palette of fluorescent probes with varying emission colors for imaging hydrogen peroxide signaling in living cells. *Journal of the American Chemical Society*. 2010;132:5906-5915

23. McCormack RN, Mendez P, Barkam S, Neal CJ, Das S, Seal S. Inhibition of nanoceria's catalytic activity due to Ce^{3+} site-specific interaction with phosphate ions. *the Journal of Physical Chemistry C* 2013;118:18992-19006.

24. Naganuma T, Traversa E. The effect of cerium valence states at cerium oxide nanoparticle surfaces on cell proliferation. *Biomaterials*. 2014;35:4441-4453

25. Grosse S, Haugland HK, Lilleng P, Ellison P, Hallan G, Hol PJ. Wear particles and ions from cemented and uncemented titanium-based hip prostheses-a histological and chemical analysis of retrieval material. *Journal of biomedical materials research. Part B, Applied biomaterials*. 2015;103:709-717

26. Gupta A, Tripathi G, Lahiri D, Balani K. Compression molded ultra high molecular weight polyethylene-hydroxyapatite-aluminum oxide-carbon nanotube hybrid composites for hard tissue replacement. *Journal of Materials Science & Technology* 2013;29:514-522.

27. Baier RE. Surface behaviour of biomaterials: The theta surface for biocompatibility. *J Mater Sci Mater Med*. 2006;17:1057-1062

28. Chigurupati S, Mughal MR, Okun E, Das S, Kumar A, McCaffery M, Seal S, Mattson MP. Effects of cerium oxide nanoparticles on the growth of keratinocytes, fibroblasts and vascular endothelial cells in cutaneous wound healing. *Biomaterials*. 2013;34:2194-2201

29. Biswas R, Sanders R. The effects of a ceo2 coating on the corrosion parameters of type 304 stainless steel. . *Journal of materials engineering and performance*. 1998;7:727-732
30. Yang Y, Han J, Ning X, Cao W, Xu W, Guo L. Controllable morphology and conductivity of electrodeposited cu(2)o thin film: Effect of surfactants. *ACS Appl Mater Interfaces*. 2014;6:22534-22543
31. Montemor MF, Pinto R, Ferreira M. Chemical composition and corrosion protection of silane films modified with ceo 2 nanoparticles. . *Electrochimica Acta* 2009;54:5179-5189.
32. Hamdy AS. Advanced nano-particles anti-corrosion ceria based sol gel coatings for aluminum alloys. *Materials Letters*. 2006;60:2633-2637
33. Ishizaki T, Masuda Y, Sakamoto M. Corrosion resistance and durability of superhydrophobic surface formed on magnesium alloy coated with nanostructured cerium oxide film and fluoroalkylsilane molecules in corrosive nacl aqueous solution. *Langmuir : the ACS journal of surfaces and colloids*. 2011;27:4780-4788
34. Wang Y, Wei M, Gao J, Hu J, Zheng Y. Corrosion process of pure magnesium in simulated body fluid. . *Materials letters*. 2008;62: 2181-2184
35. Huang CH, Lai JJ, Wei TY, Chen YH, Wang X, Kuan SY, Huang JC. Improvement of bio-corrosion resistance for ti42zr40si15ta3 metallic glasses in simulated body fluid by annealing within supercooled liquid region. *Mater Sci Eng C Mater Biol Appl*. 2015;52:144-150
36. Kim W-H, Kim M-K, Maeng W, Gatineau J, Pallem V, Dussarrat C, Noorin A, Thompson DD, Chu S, Kim H. Growth characteristics and film properties of cerium dioxide prepared by plasma-enhanced atomic layer deposition. *Journal of The Electrochemical Society* 2011;158:G169-G172

37. Korsvik C, Patil S, Seal S, Self WT. Superoxide dismutase mimetic properties exhibited by vacancy engineered ceria nanoparticles. *Chemical communications*. 2007;1056-1058
38. Heckert EG, Karakoti AS, Seal S, Self WT. The role of cerium redox state in the sod
5 mimetic activity of nanoceria. *Biomaterials*. 2008;29:2705-2709
39. Pirmohamed T, Dowding JM, Singh S, Wasserman B, Heckert E, Karakoti AS, King
JE, Seal S, Self WT. Nanoceria exhibit redox state-dependent catalase mimetic
activity. *Chemical communications*. 2010;46:2736-2738
40. Dowding JM, Dosani T, Kumar A, Seal S, Self WT. Cerium oxide nanoparticles
10 scavenge nitric oxide radical (no). *Chemical communications*. 2012;48:4896-4898
41. Dowding JM, Das S, Kumar A, Dosani T, McCormack R, Gupta A, Sayle TX, Sayle
DC, von Kalm L, Seal S, Self WT. Cellular interaction and toxicity depend on
physicochemical properties and surface modification of redox-active nanomaterials.
ACS nano. 2013;7:4855-4868
- 15 42. Walkey C, Das S, Seal S, Erlichman J, Heckman K, Ghibelli L, Traversa E, McGinnis
JF, Self WT. Catalytic properties and biomedical applications of cerium oxide
nanoparticles. *Environ Sci Nano*. 2015;2:33-53
43. Cai X, Seal S, McGinnis JF. Sustained inhibition of neovascularization in vldlr-/-
mice following intravitreal injection of cerium oxide nanoparticles and the role of the
20 ask1-p38/jnk-nf-kappab pathway. *Biomaterials*. 2014;35:249-258
44. Cai X, Yodoi J, Seal S, McGinnis JF. Nanoceria and thioredoxin regulate a common
antioxidative gene network in tubby mice. *Advances in experimental medicine and
biology*. 2014;801:829-836
45. Heckman KL, DeCoteau W, Estevez A, Reed KJ, Costanzo W, Sanford D, Leiter JC,
25 Clauss J, Knapp K, Gomez C, Mullen P, Rathbun E, Prime K, Marini J, Patchefsky J,

Patchefsky AS, Hailstone RK, Erlichman JS. Custom cerium oxide nanoparticles protect against a free radical mediated autoimmune degenerative disease in the brain. . *ACS nano*. 2013;7:10582-10596

46. Hirst SM, Karakoti A, Singh S, Self W, Tyler R, Seal S, Reilly CM. Bio-distribution and *in vivo* antioxidant effects of cerium oxide nanoparticles in mice. *Environmental toxicology*. 2013;28:107-118

47. Hirst SM, Karakoti AS, Tyler RD, Sriranganathan N, Seal S, Reilly CM. Anti-inflammatory properties of cerium oxide nanoparticles. *Small*. 2009;5:2848-2856

48. Lung S, Cassee FR, Gosens I, Campbell A. Brain suppression of ap-1 by inhaled diesel exhaust and reversal by cerium oxide nanoparticles. *Inhal Toxicol*. 2014;26:636-641

49. Rocca A, Moscato S, Ronca F, Nitti S, Mattoli V, Giorgi M, Ciofani G. Pilot *in vivo* investigation of cerium oxide nanoparticles as a novel anti-obesity pharmaceutical formulation. *Nanomedicine : nanotechnology, biology, and medicine*. 2015;11:1725-1734

50. Dowding JM, Song W, Bossy K, Karakoti A, Kumar A, Kim A, Bossy B, Seal S, Ellisman MH, Perkins G, Self WT, Bossy-Wetzel E. Cerium oxide nanoparticles protect against abeta-induced mitochondrial fragmentation and neuronal cell death. *Cell death and differentiation*. 2014;21:1622-1632

51. Abu-Amer Y, Darwech I, Clohisy JC. Aseptic loosening of total joint replacements: Mechanisms underlying osteolysis and potential therapies. *Arthritis research & therapy*. 2007;9 Suppl 1:S6

52. Cronin JG, Turner ML, Goetze L, Bryant CE, Sheldon IM. Toll-like receptor 4 and myd88-dependent signaling mechanisms of the innate immune system are essential

for the response to lipopolysaccharide by epithelial and stromal cells of the bovine endometrium. *Biol Reprod.* 2012;86:51

53. Kruger B, Yin N, Zhang N, Yadav A, Coward W, Lal G, Zang W, P SH, Bromberg JS, Murphy B, Schroppel B. Islet-expressed tlr2 and tlr4 sense injury and mediate
5 early graft failure after transplantation. *European journal of immunology.* 2010;40:2914-2924

54. Soloviev A, Schwarz EM, Kuprash DV, Nedospasov SA, Puzas JE, Rosier RN, O'Keefe RJ. The role of p105 protein in nfkappab activation in ana-1 murine macrophages following stimulation with titanium particles. *Journal of orthopaedic
10 research : official publication of the Orthopaedic Research Society.* 2002;20:714-722

55. Platt ID, Josse AR, Kendall CW, Jenkins DJ, El-Sohemy A. Postprandial effects of almond consumption on human osteoclast precursors--an ex vivo study. *Metabolism.* 2011;60:923-929

56. Katagiri M, Hakeda Y, Chikazu D, Ogasawara T, Takato T, Kumegawa M, Nakamura
15 K, Kawaguchi H. Mechanism of stimulation of osteoclastic bone resorption through gas6/tyro 3, a receptor tyrosine kinase signaling, in mouse osteoclasts. *The Journal of biological chemistry.* 2001;276:7376-7382

57. Cheng SL, Ramachandran B, Behrmann A, Shao JS, Mead M, Smith C, Krcma K, Bello Arredondo Y, Kovacs A, Kapoor K, Brill LM, Perera R, Williams BO, Towler
20 DA. Vascular smooth muscle lrp6 limits arteriosclerotic calcification in diabetic ldlr-/- mice by restraining noncanonical wnt signals. *Circulation research.* 2015;117:142-156

58. Cheng SL, Shao JS, Cai J, Sierra OL, Towler DA. Msx2 exerts bone anabolism via canonical wnt signaling. *The Journal of biological chemistry.* 2008;283:20505-20522

59. Shao JS, Cheng SL, Charlton-Kachigian N, Loewy AP, Towler DA. Teriparatide (human parathyroid hormone (1-34)) inhibits osteogenic vascular calcification in diabetic low density lipoprotein receptor-deficient mice. *The Journal of biological chemistry*. 2003;278:50195-50202
- 5 60. Hirakawa K, Bauer TW, Stulberg BN, Wilde AH. Comparison and quantitation of wear debris of failed total hip and total knee arthroplasty. *J Biomed Mater Res*. 1996;31:257-263
61. Ponnusamy M, Liu N, Sellamuthu R, Zhao TC, Mao H, Zhuang S. Autophagy protects against necrotic renal epithelial cell-induced death of renal interstitial
10 fibroblasts. *American journal of physiology. Renal physiology*. 2012;303:F83-91
62. Keegan GM, Learmonth ID, Case CP. Orthopaedic metals and their potential toxicity in the arthroplasty patient: A review of current knowledge and future strategies. *The Journal of bone and joint surgery. British volume*. 2007;89:567-573
63. Yadav VR, Prasad S, Sung B, Aggarwal BB. The role of chalcones in suppression of
15 nf-kappab-mediated inflammation and cancer. *Int Immunopharmacol*. 2011;11:295-309
64. Noor Z, Kania N, Setiawan B. Tibia bone properties at different time course of ovariectomized rats. *J Diabetes Metab Disord*. 2014;13:91
65. Faienza MF, Ventura A, Piacente L, Ciccarelli M, Gigante M, Gesualdo L, Colucci S,
20 Cavallo L, Grano M, Brunetti G. Osteoclastogenic potential of peripheral blood mononuclear cells in cleidocranial dysplasia. *Int J Med Sci*. 2014;11:356-364
66. Lai CF, Shao JS, Behrmann A, Krcchma K, Cheng SL, Towler DA. Tnfr1-activated reactive oxidative species signals up-regulate osteogenic msx2 programs in aortic myofibroblasts. *Endocrinology*. 2012;153:3897-3910

67. Cheng SL, Shao JS, Behrmann A, Krcma K, Towler DA. Dkk1 and msx2-wnt7b signaling reciprocally regulate the endothelial-mesenchymal transition in aortic endothelial cells. *Arterioscler Thromb Vasc Biol.* 2013;33:1679-1689
68. Olivares-Navarrete R, Raines AL, Hyzy SL, Park JH, Hutton DL, Cochran DL, Boyan BD, Schwartz Z. Osteoblast maturation and new bone formation in response to titanium implant surface features are reduced with age. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research.* 2012;27:1773-1783
69. Kayal RA, Tsatsas D, Bauer MA, Allen B, Al-Sebaei MO, Kakar S, Leone CW, Morgan EF, Gerstenfeld LC, Einhorn TA, Graves DT. Diminished bone formation during diabetic fracture healing is related to the premature resorption of cartilage associated with increased osteoclast activity. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research.* 2007;22:560-568
70. Zou H, Zhao X, Sun N, Zhang S, Sato T, Yu H, Chen Q, Weber HP, Dard M, Yuan Q, Lanske B. Effect of chronic kidney disease on the healing of titanium implants. *Bone.* 2013;56:410-415
71. Seong WJ, Grami S, Jeong SC, Conrad HJ, Hodges JS. Comparison of push-in versus pull-out tests on bone-implant interfaces of rabbit tibia dental implant healing model. *Clin Implant Dent Relat Res.* 2013;15:460-469
72. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: The arrive guidelines for reporting animal research. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society.* 2012;20:256-260
73. Bolli R. The new circulation research: A manifesto. *Circulation research.* 2010;106:216-226

74. Windahl SH, Lagerquist MK, Andersson N, Jochems C, Kallkopf A, Hakansson C, Inzunza J, Gustafsson JA, van der Saag PT, Carlsten H, Pettersson K, Ohlsson C. Identification of target cells for the genomic effects of estrogens in bone. *Endocrinology*. 2007;148:5688-5695
- 5 75. Cheng SL, Behrmann A, Shao JS, Ramachandran B, Krchma K, Bello Arredondo Y, Kovacs A, Mead M, Maxson R, Towler DA. Targeted reduction of vascular msx1 and msx2 mitigates arteriosclerotic calcification and aortic stiffness in ldlr-deficient mice fed diabetogenic diets. *Diabetes*. 2014;63:4326-4337
- 10 76. Wohl GR, Towler DA, Silva MJ. Stress fracture healing: Fatigue loading of the rat ulna induces upregulation in expression of osteogenic and angiogenic genes that mimic the intramembranous portion of fracture repair. *Bone*. 2009;44:320-330
- 15 77. Schmidt A, Harada S, Kimmel DB, Bai C, Chen F, Rutledge SJ, Vogel RL, Scafonas A, Gentile MA, Nantermet PV, McElwee-Witmer S, Pennypacker B, Masarachia P, Sahoo SP, Kim Y, Meissner RS, Hartman GD, Duggan ME, Rodan GA, Towler DA, Ray WJ. Identification of anabolic selective androgen receptor modulators with reduced activities in reproductive tissues and sebaceous glands. *The Journal of biological chemistry*. 2009;284:36367-36376
- 20 78. Scafonas A, Reszka AA, Kimmel DB, Hou XS, Su Q, Birzin ET, Kim S, Chen HY, Tan Q, Roher SP, Dinunno F, Hammond ML, Rodan GA, Towler DA, Schmidt A. Agonist-like serum effects on estrogen-mediated repression of mmp1 promoter activity predict *in vivo* effects on bone and uterus. *J Steroid Biochem Mol Biol*. 2008;110:197-206
- 25 79. Troiano NW, Ciovacco WA, Kacena MA. The effects of fixation and dehydration on the histological quality of undecalcified murine bone specimens embedded in methylmethacrylate. *J Histotechnol*. 2009;32:27-31

80. Dempster DW, Compston JE, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR, Parfitt AM. Standardized nomenclature, symbols, and units for bone histomorphometry: A 2012 update of the report of the asbmr histomorphometry nomenclature committee. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2013;28:2-17
81. Shao JS, Cheng SL, Pingsterhaus JM, Charlton-Kachigian N, Loewy AP, Towler DA. Mx2 promotes cardiovascular calcification by activating paracrine wnt signals. *The Journal of clinical investigation*. 2005;115:1210-1220
82. Silva MJ. Biomechanics of osteoporotic fractures. *Injury*. 2007;38 Suppl 3:S69-76
83. Jepsen KJ, Silva MJ, Vashishth D, Guo XE, van der Meulen MC. Establishing biomechanical mechanisms in mouse models: Practical guidelines for systematically evaluating phenotypic changes in the diaphyses of long bones. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2015;30:951-966
84. Jilka RL, Almeida M, Ambrogini E, Han L, Roberson PK, Weinstein RS, Manolagas SC. Decreased oxidative stress and greater bone anabolism in the aged, when compared to the young, murine skeleton with parathyroid hormone administration. *Aging cell*. 2010;9:851-867
85. Almeida M, Ambrogini E, Han L, Manolagas SC, Jilka RL. Increased lipid oxidation causes oxidative stress, increased peroxisome proliferator-activated receptor-gamma expression, and diminished pro-osteogenic wnt signaling in the skeleton. *The Journal of biological chemistry*. 2009;284:27438-27448
86. Yang X, Ricciardi BF, Dvornzhinskiy A, Brial C, Lane Z, Bhimani S, Burket JC, Hu B, Sarkisian AM, Ross FP, van der Meulen MC, Bostrom MP. Intermittent

parathyroid hormone enhances cancellous osseointegration of a novel murine tibial implant. *The Journal of bone and joint surgery. American volume*. 2015;97:1074-1083

87. Nyazee HA, Finney KM, Sarikonda M, Towler DA, Johnson JE, Babcock HM.

5 Diabetic foot osteomyelitis: Bone markers and treatment outcomes. *Diabetes Res Clin Pract*. 2012;97:411-417

88. Novack DV, Teitelbaum SL. The osteoclast: friend or foe? *Annu Rev pathmechdis Mech Dis*. 2008;3:457-84.

10

15

CLAIMS

What is claimed is:

1. An implant comprising a substrate having one or more nanoceria coatings coated at least partially thereon, wherein the one or more nanoceria coatings comprise surface cerium having a 3+/4+ oxidation state ratio such that the one or more nanoceria coatings exhibit catalase mimetic activity, superoxide dismutase mimetic activity, or both.
2. The implant of claim 1, wherein the surface roughness of the coating is 30 nm to 40 nm of Ra.
3. The implant of claim 1 wherein the surface cerium 3+/4+ oxidation state ratio is such that osteolysis at the surface is reduced.
4. The implant of claim 1, wherein the one or more nanoceria coatings have a root mean square surface roughness (Rq) between 40 nm to 85 nm.
5. The implant of claim 1, wherein the substrate comprises at least one or a combination of metal, ceramic, or plastic.
6. The implant of claim 1, further comprising an intermediate layer between the substrate and the nanoceria coating.
7. The implant of claim 1, wherein the nanoceria coating has a thickness of at least 200 nm.

8. The implant of claim 1, wherein the nanoceria coating has a thickness between 200 nm and 500 nm.

9. A method of making an implant comprising the steps of:

5 obtaining a substrate comprising materials comprised in whole or in part of metal, ceramic, plastic, or a composite;

depositing a nanoceria coating on at least a portion of the substrate, the nanoceria coating having a predetermined surface roughness parameter and a surface cerium 3+/4+ oxidation state ratio such that said nanoceria coating exhibits catalase
10 mimetic activity, superoxide dismutase mimetic activity, or both.

10. The method of claim 9, wherein osteolysis at the surface of the nanoceria coating is reduced.

15 11. The method of claim 9, wherein the coating step is performed using dip coating under heat in an inert atmosphere.

12. The method of claim 9, where the substrate is comprised of a conductive material and the coating step is performed using electrophoretic deposition.

20

13. The method of claim 9, wherein the nanoceria coating deposited has a thickness of at least 200 nm.

14. The method of claim 13, wherein the nanoceria coating has a thickness between 200 nm
25 and 500 nm.

15. The method of claim 9, wherein the coating has an average roughness between 30-40 nm.

16. The method of claim 9, wherein the coating has a root mean square roughness between 40 and 85 nm.

5

17. A method of reducing osteolysis associated with an implant, the method comprising administering nanoceria in proximity to a bone-implant interface, wherein the nanoceria comprises catalase activity, superoxide dismutase activity, or both.

10 18. A method for forming a coating comprising the steps of:

providing a substrate; and

electrophoretically forming the coating using a dispersion of ceria nanoparticles;

15 such that a coating comprising nanoceria is formed on at least a portion the substrate.

19. The method of claim 18, wherein the electrophoretic forming step is carried out by applying a voltage having a DC component and/or an AC component to the substrate and at least one counter-electrode, wherein the voltage is continuous, pulsed, or arbitrarily increasing or decreasing with time.

20

20. The method of claim 19, wherein at least one counter-electrode comprises two counter-electrodes.

21. The method of claim 18, wherein the cerium oxide nanoparticles comprise cerium in the 3+ oxidation state or 4+ oxidation state, or both.

22. The method of claim 18, further comprising after the nanoceria coating has been formed, heating the substrate to 200-450°C for 1-2.5 hr.

23. The method as recited in claim 18, wherein one electrode is a Ti electrode and two electrodes are nonconsumable counter-electrodes.

5 24. A coating by the process of claim 18, further characterized by nanoceria having a surface cerium 3+/4+ oxidation state ratio such that the coating exhibits catalase mimetic activity, superoxide dismutase mimetic activity, or both.

25. The coating of claim 25, comprising a coating for a prosthesis.

10 26. The implant of claim 1, wherein the implant is used for contact with mammalian tissue.

27. The implant of claim 26, wherein the mammalian tissue is bone tissue.

28. The implant of claim 26, wherein the mammalian tissue is human tissue.

29. A method of conducting an orthopedic procedure, the method comprising

obtaining an implant of claim 1; and

15 implanting the implant in a subject in need thereof.

30. The method of claim 29, wherein the implanting comprises contacting the implant with bone tissue.

31. A method of reducing osteolysis in a subject in need, the method comprising administering nanoceria comprising catalase activity, superoxide dismutase activity, or both,
20 to an injury, defect or disease site of bone in the subject.

32. A bone paste comprising nanoceria comprising catalase activity, superoxide dismutase activity, or both, and at least one of an osteoinductive or osteoconductive component.
33. The bone paste of claim 32, further comprising a suitable carrier component.
- 5 34. The bone paste of claims 32 or 33 further comprising a bone paste additive component.

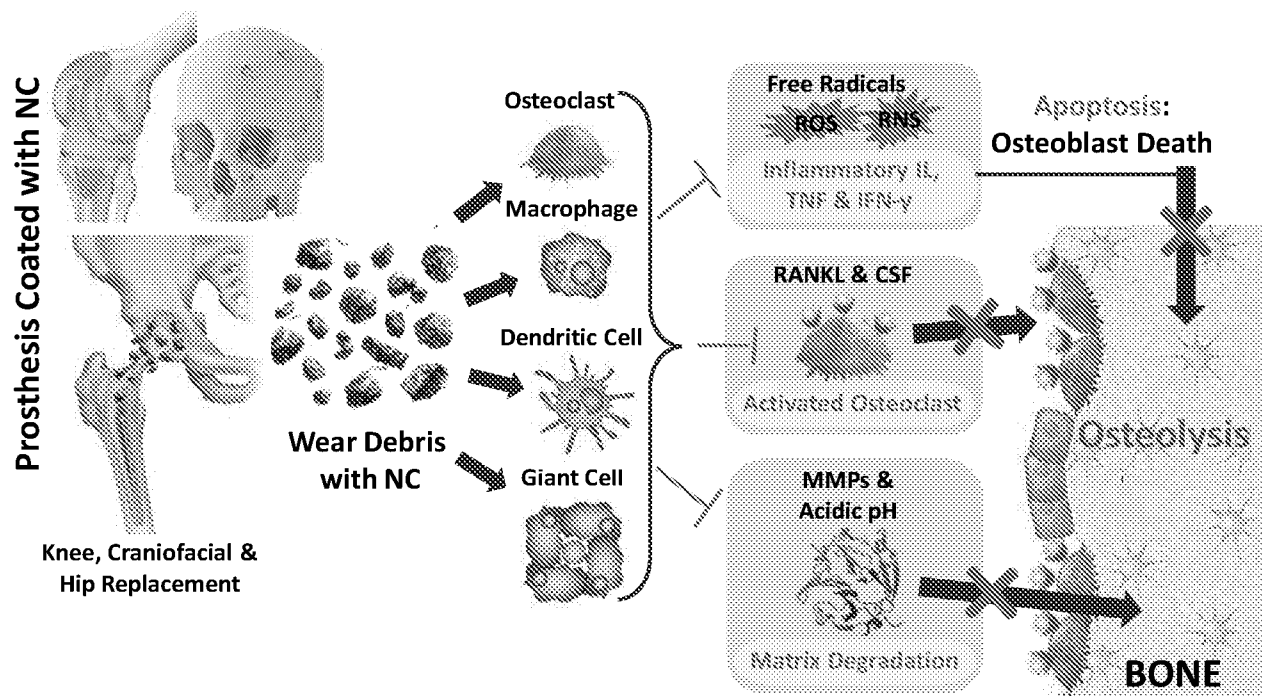
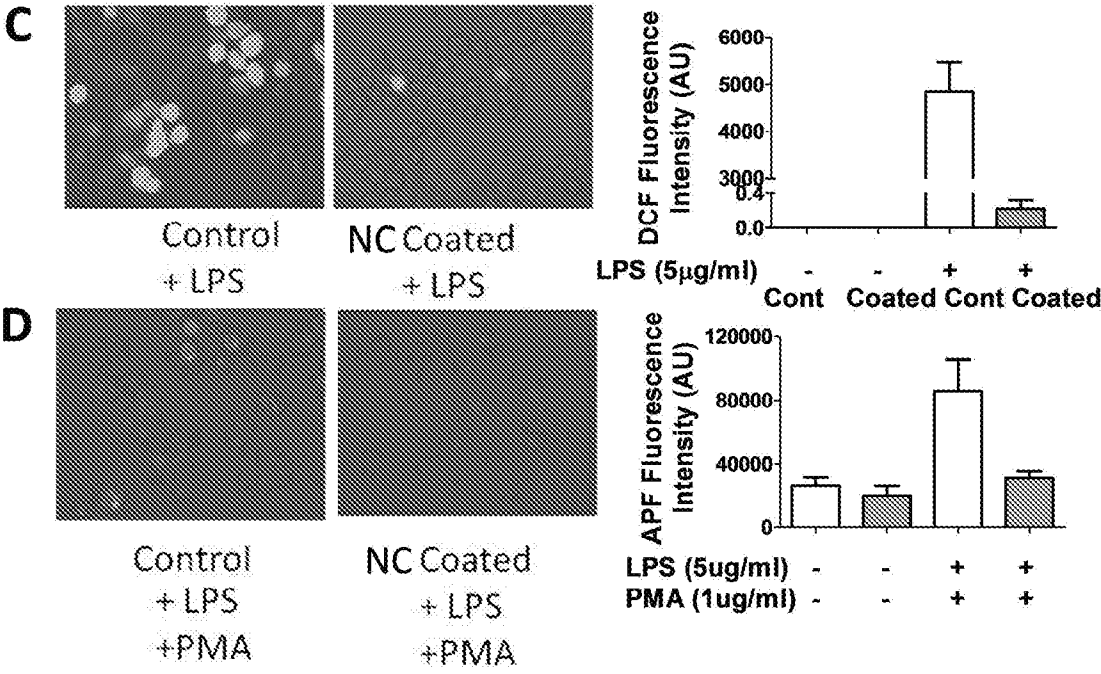
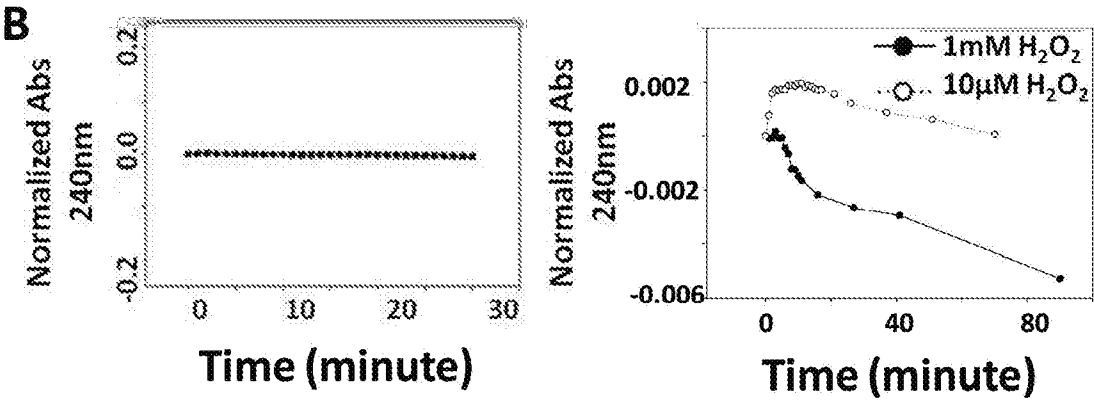
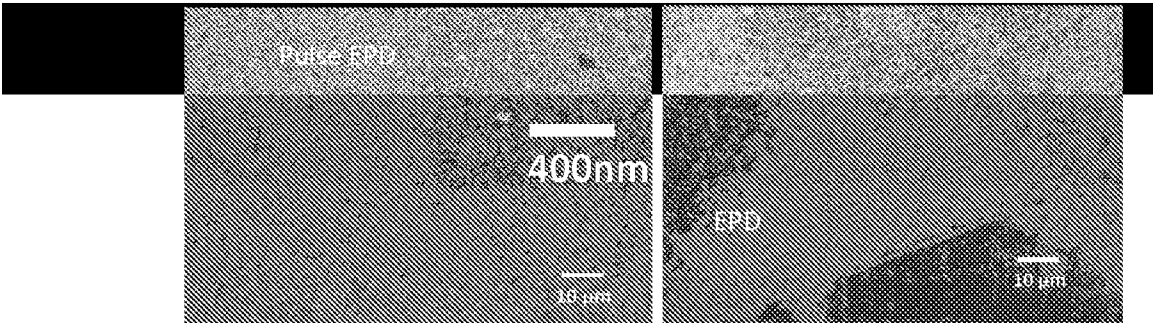


Figure 1



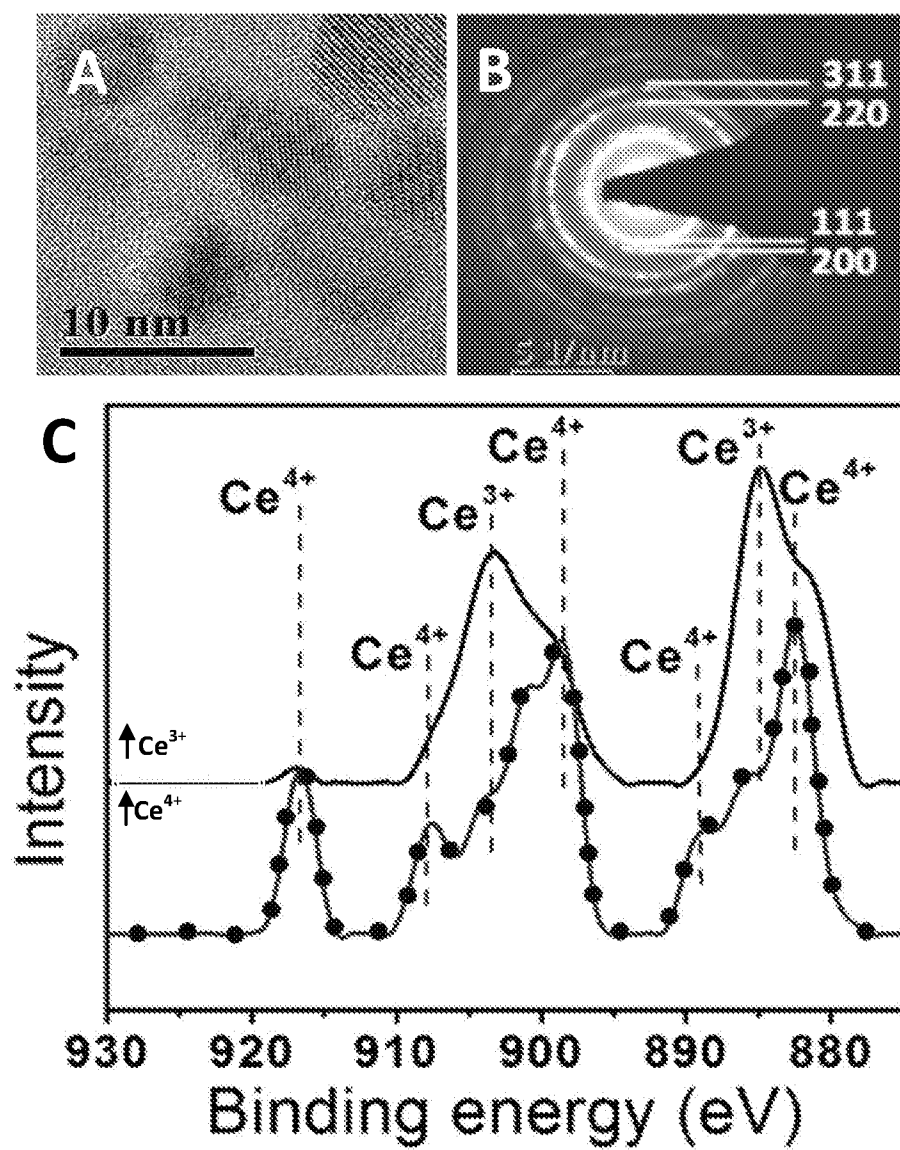


Figure 3

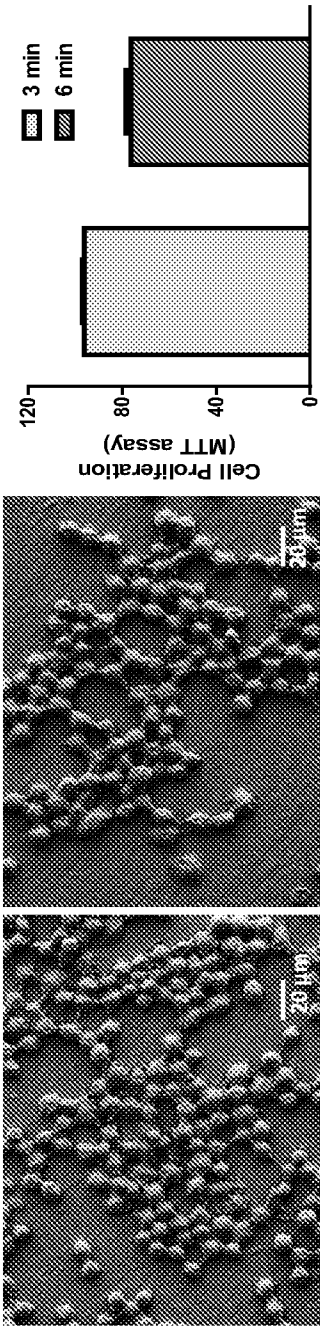


Figure 4

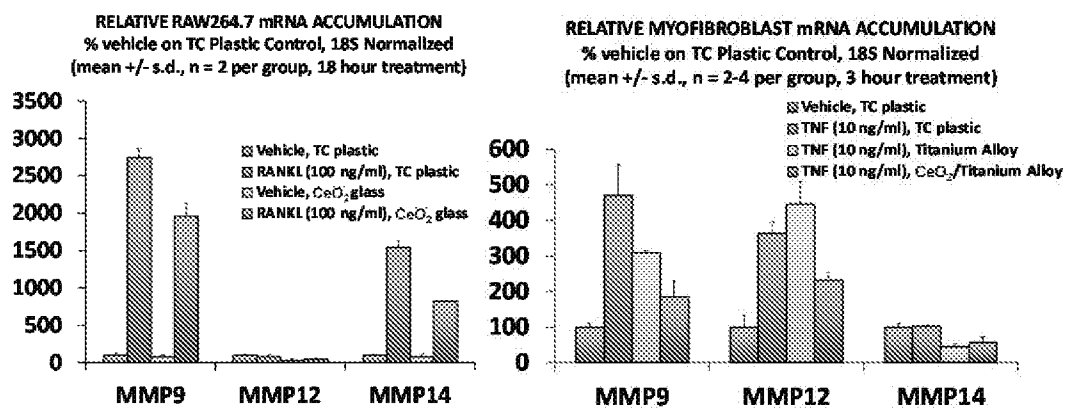


Figure 5

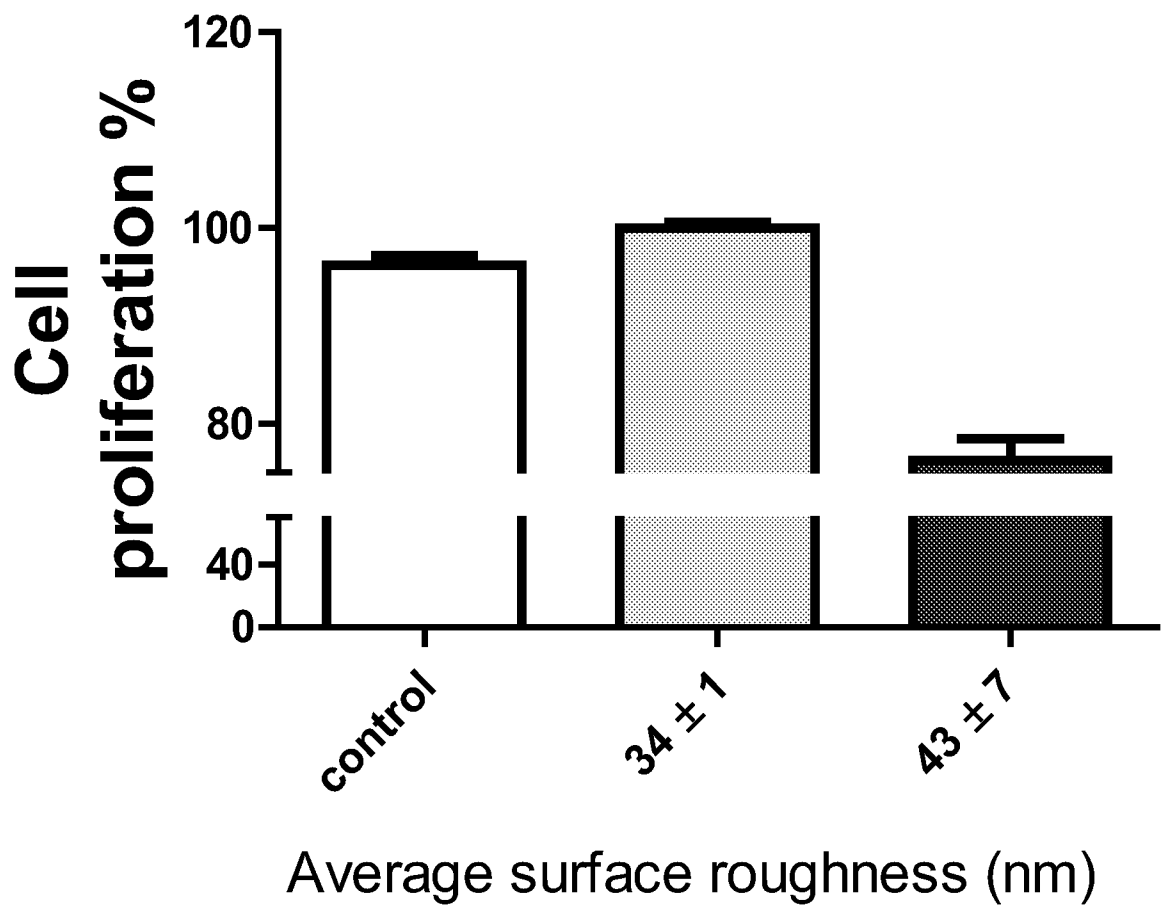


Figure 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/055683**A. CLASSIFICATION OF SUBJECT MATTER****A61L 27/30(2006.01)i, A61L 27/04(2006.01)i, A61L 27/10(2006.01)i, A61L 27/14(2006.01)i, C25D 15/00(2006.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61L 27/30; A61K 9/14; A01N 59/16; A61K 47/02; A61K 35/12; A61K 9/16; A61K 33/24; A61L 27/04; A61L 27/10; A61L 27/14; C25D 15/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & keywords: osteolysis, reduced, implant, coating, cerium oxide, nanoceria, catalase, superoxide dismutase**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 7534453 B1 (RZIGALINSKI, B. A. et al.) 19 May 2009 See claim 1; column 4, lines 2-5.	1-28,32-34
Y	NAGANUMA, T., et al., "The effect of cerium valence states at cerium oxide nanoparticle surfaces on cell proliferation", Biomaterials, vol. 35, Issue 15, pages 4441-4453, 31 May 2014 See abstract; page 4442, Introduction.	1-17,24-28,32-34
Y	NG, W.F., et al., "Cerium-based coating for enhancing the corrosion resistance of bio-degradable Mg implants", Materials Chemistry and Physics, vol. 119, Issue 3, pages 384-388, 15 February 2010 See page 384, Introduction; page 385, Sample preparation.	11-12,18-25
A	WO 2014-134286 A2 (UNIVERSITY OF CENTRAL FLORIDA RESEARCH FOUNDATION, INC.) 04 September 2014 See entire document.	1-28,32-34
A	US 2011-0135740 A1 (SUGAYA, K. et al.) 09 June 2011 See entire document.	1-28,32-34



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

09 February 2017 (09.02.2017)

Date of mailing of the international search report

16 February 2017 (16.02.2017)

Name and mailing address of the ISA/KR

International Application Division

Korean Intellectual Property Office

189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea



Facsimile No. +82-42-481-8578

Authorized officer

CHO, Ki Yun

Telephone No. +82-42-481-5655



INTERNATIONAL SEARCH REPORTInternational application No.
PCT/US2016/055683**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 29-31
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 29-31 pertain to methods for treatment of the human body and thus relate to a subject-matter which this International Searching Authority is not required to search under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv).
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2016/055683Patent document
cited in search reportPublication
datePatent family
member(s)Publication
date

US 7534453 B1

19/05/2009

None

WO 2014-134286 A2

04/09/2014

US 2016-0015741 A1

21/01/2016

US 2011-0135740 A1

09/06/2011

US 2007-0123996 A1

31/05/2007

US 7888119 B2

15/02/2011

US 8153158 B2

10/04/2012