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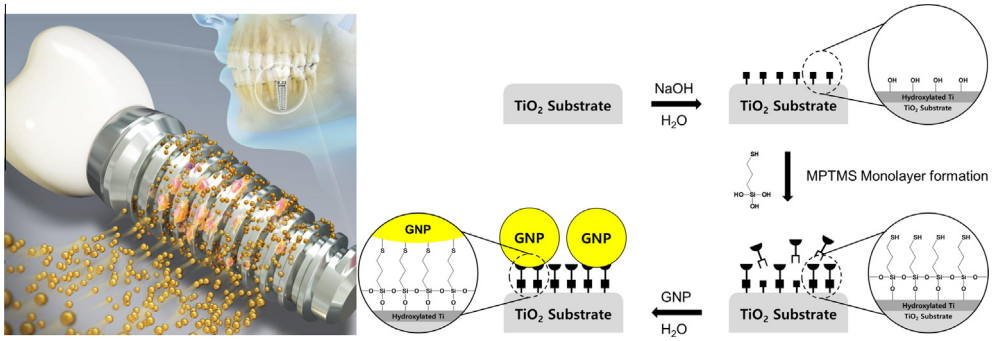
Titanium dental implants surface-immobilized with gold nanoparticles as osteoinductive agents for rapid osseointegration

Dong Nyoung Heo [a,b,1], Wan-Kyu Ko [a,1], Hak Rae Lee [c], Sang Jin Lee [a], Donghyun Lee [a], Soong Ho Um [d], Jung Haeng Lee [e], Yi-Hyung Woo [e], Lijie Grace Zhang [b], Deok-Won Lee [f,*], Il Keun Kwon [a,*]

[a] Department of Dental Materials, School of Dentistry, Kyung Hee University, Seoul 130-701, Republic of Korea
[b] Department of Mechanical and Aerospace Engineering, The George Washington University, DC 20052, USA
[c] Department of Chemical and Biomolecular Engineering and KI for Nano Century, Korea Advanced Institute Science and Technology, Daejeon 305-701, Republic of Korea
[d] School of Chemical Engineering & SKKU Advanced Institute of Nanotechnology, Sungkyunkwan University, Suwon, Gyeonggi-do 440-746, Republic of Korea
[e] Department of Prosthodontics, School of Dentistry, Kyung Hee University, Seoul 130-701, Republic of Korea
[f] Department of Oral and Maxillofacial Surgery, Kyung Hee University Dental Hospital at Gang-dong, Seoul 134-727, Republic of Korea

GRAPHICAL ABSTRACT



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ABSTRACT

Gold nanoparticles (GNPs) are quite attractive materials for use as osteogenic agents due to their potential effects on the stimulation of osteoblast differentiation. In this study, an osseo-integrated titanium (Ti) implant surface coated with GNPs was used for promotion of bone regeneration. We prepared a silanized Ti surface by chemical treatment of (3-Mercaptopropyl) trimethoxysilane (MPTMS) and immobilized the GNP layer (Ti–GNP) on their surfaces via Au–S bonding. The GNP layer is uniformly immobilized on the surface and the layer covers the titanium oxide surface well, as confirmed by scanning electron microscopy (SEM) and atomic force microscopy (AFM). The Ti–GNP was used to investigate the effectiveness of this system both in vitro and in vivo. The in vitro results showed that the Ti–GNP significantly enhances the osteogenic differentiation with increased mRNA expression of osteogenic differentiation specific genes in human adipose-derived stem cells (ADSCs). Furthermore, the in vivo results showed that Ti–GNP had a significant influence on the osseous interface formation. Through these in vitro and vivo tests, we found that Ti–GNP can be useful as osseo-integration inducing dental implants for formation of an osseous interface and maintenance of nascent bone formation.

* Corresponding authors.
E-mail addresses: verycutebear@hanmail.net (D.-W. Lee), kwoni@khu.ac.kr (I.K. Kwon).
[1] Dong Nyoung Heo and Wan-Kyu Ko both authors contributed equally to this work.

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1. Introduction

Titanium (Ti) has been widely used as a dental material for biomedical devices and implants due to its many advantages. These advantages include high mechanical strength, corrosion resistance, and biocompatibility [1,2]. Especially, various shapes of dental implants, including the screw and abutment types, are mostly made of titanium and its alloys [3]. In dental medicine, these are used as an artificial tooth root to replace failed teeth. Current trends in clinical dental sciences are slanted toward the development of an osseointegrated implant surface with nano- and micro-scale topographies for successful bone regeneration and healing in dental and orthopedic applications [4,5]. To accomplish this, recently developed approaches are summarized as follows, (1) physical treatments such as sand blasting, ion beam deposition, and compaction of nanoparticles, (2) chemical treatments such as acid etching, anodizing, alkali treatment, and peroxidation, and (3) chemical conjugation of osteoconductive or osteoinductive biomolecules [6]. The main goals of most of the approaches mentioned above are to promote the osseointegration and to maintain its formation on the surface-treated dental implants.

Gold nanoparticles (GNPs) have been extensively used in a broad range of applications such as targeted delivery of drugs, peptides, and genes, diagnosis, biosensing, molecular imaging, and tissue engineering. This is due to their unique optical, electrical, chemical, and structural properties [7–10]. In the tissue engineering field, it was reported that GNPs are quite attractive materials for use as osteogenic agents in order to achieve bone tissue regeneration. Many researchers found that GNPs have a positive effect on osteogenic differentiation of osteo-progenitor cells after intracellular uptake [11–13]. In particular, the osteogenic differentiation of mesenchymal stem cells into osteoblasts was greatly enhanced when cultured in the presence of 30 or 50 nm sized GNPs [14]. Also, the effects of GNPs as osteogenic agents were confirmed by animal studies. It was reported that GNPs embedded in a hydrogel can enhance new bone formation in the defect sites of rabbits, and have similar effectiveness as compared with a bone morphogenic protein (BMPs) loaded hydrogel used as a positive control [15]. Therefore, GNPs are quite attractive materials in the field of bone tissue engineering when they are introduced on the surface of dental implants. In order for GNPs to be utilized as surface-enhancing bioactive agents on implants, the GNPs should be easily applied and stably attached to the implant surface in a manner which maintains their efficacy after implantation.

In this study, we used human adipose-derived stem cells (ADSCs) that can be readily differentiated to several types of cells such as osteoblasts, adipocytes, chondrocytes, and myoblasts [16]. Especially, ADSCs were widely used in dental area such as periodontal tissue regeneration and bone tissue engineering [17–20].

In view of the importance of the above studies, we have designed and prepared osseointegrated Ti implants surface-functionalized with GNPs (Ti–GNP) for use as osteoinductive agent (Fig. 1). The GNPs were conjugated on the surface of silanized Ti implants by binding via Au–S bonds. The surface chemical characterization and distribution of GNPs on the Ti surfaces were determined by X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), and atomic force microscopy (AFM). The Ti–GNP surfaces were evaluated for their capacity to enhance the osteogenic differentiation of ADSCs. Additionally, the effectiveness of Ti–GNP surfaces for formation of the osseous interface and maintenance of their formation was evaluated under in vivo conditions.

2. Materials and methods

2.1. Materials

Titanium (Ti) disks meeting ASTM F67 grade 4 were obtained from Biotem (Sungnam, Republic of Korea). The chemicals used in the synthesis of gold nanoparticles (GNPs) [chloroauric acid ($HAuCl_4$) and trisodium citrate] and for surface silanization [(3-Mercaptopropyl) trimethoxysilane (MPTMS)] were purchased from Sigma–Aldrich (St. Louis, MO). Human adipose-derived stem cells (ADSCs) were purchased from Invitrogen (Carlsbad, CA, USA) and cultured in MesenPRO RS™ medium supplemented with MesenPRO RS™ growth supplement. Osteogenic medium comprising of Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS, GIBCO, Gran Island, NY), 1% penicillin–streptomycin (PS, GIBCO), 10 mM – β-glycerol phosphate (Sigma–Aldrich), 300 μM ascorbic acid (Sigma–Aldrich) and 0.1 μM dexamethasone (Sigma–Aldrich) was used for cell differentiation. All reagents were used as received without further purification.

2.2. GNPs synthesis

GNPs were synthesized by the reduction of chloroauric acid with trisodium citrate as described previously [21]. The GNPs solution was used without further purification in all experiments.

2.3. Preparation of GNPs immobilized on Ti

Ti disks with a diameter of 10 mm and a thickness of 2 mm were used. The bare Ti disks were polished and ultrasonically cleaned three times in hexane, acetone, ethanol and deionized water (DW) for 5 min each, then dried a N_2 stream. The cleaned Ti disks were immersed in a 2.5 N NaOH solution at 80 °C for 24 h, then rinsed thoroughly with deionized water and ultrasonically cleaned with DW (labeled as TiOH). Subsequently, TiOH disks were immersed into 2% MPTMS solution in toluene at 60 °C for 24 h. The disks were taken out, then successively washed with toluene, ethanol, DW, and dried in a N_2 stream (labeled as TiSH). Finally, the TiSH disks were immersed into the GNP solution at 37 °C for 24 h, and then rinsed thoroughly with deionized water and ultrasonically cleaned with DW (labeled as Ti–GNP).

2.4. Surface characterization

X-ray photoelectron spectroscopy (XPS) was used to examine the surface chemical compositions of various forms of Ti. The XPS spectra were collected on a K-Alpha (Thermo Scientific, UK) configured with a monochromatic Al Kα X-ray source, a spot size of 400 μm, a flood gun to counter charging effects, and an ultrahigh vacuum. The chemical elements present on the samples were identified from the survey spectra. The survey scans were measured from 0 eV to 1400 eV. The surface morphologies of various forms of Ti were characterized with scanning electron microscope (SEM) and atomic force microscope (AFM). SEM (S2300, Hitachi, Japan) measurements were carried out after coating with platinum using a sputter-coater. AFM measurements were made with a PUCOStation STD (NANOS® AFM system, NanoInk, Inc., USA) operating in dynamic mode with a scanner (NANOS® 100) at room temperature. The water contact angles measurement of Ti, TiOH, TiSH, and Ti–GNP was carried out by video contact angle instrument (Phoenix 150, SEO, Korea) at room temperature, and the contact angles were calculated on each surface. All measurements were carried out five times.