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Natural bone-like biomimetic surface modification of titanium

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ABSTRACT

An implantable metallic surface consisting of titanium (Ti) was modified with natural bone-mimicking CNT–Gelatin–HA nanohybrids to create a new surface with similar properties to the surrounding bone tissue in terms of the chemical constitution, nanotopography, wettability, and biocompatibility. The biomimetic surface modification was achieved through the covalent immobilization of carbon nanotubes (CNTs) onto the Ti surface, the covalent tethering of gelatin molecules onto the CNT surface, and then the deposition of hydroxyl apatite (HA) crystals onto the gelatin-tethered CNTs in SBF solution. The SEM microscopic images demonstrated that the modified Ti surface continually maintained a fibrous structure of CNTs, but that the CNT fibers were hybridized with gelatin and HA in a multi-core-shell structure of similar constitution to that of the collagen fibers of natural bone. The new surface of the Ti substrates showed significantly higher mechanical properties and favorable wettability and biocompatibility.

1. Introduction

Titanium, as one of the implantable metallic materials, has been successfully used in a variety of clinical applications, including surgical implements and orthopedic and dental implants, because of its biocompatibility, high corrosion resistance, and load-bearing properties [1–4]. However, it is well known that the titanium surface without modification is unsuitable for osseointegration, because titanium cannot form chemical bonds with natural bone, thus leading to the loosening of the implant–native bone interface and slow healing [5,6]. In order to realize suitable osseointegration of the metal implant in the surrounding tissue, various surface modifications, such as physicochemical, morphological, and biochemical changes, have been carried out [7–9]. Moreover, dental implants could be improved by the surface immobilization of bioactive materials such as bioglass [10,11], or hydroxyl apatite (HA) [12–16], because the bioactive materials coated on the surface of an implant could more intensively stimulate osteogenic cell growth, proliferation and differentiation than the titanium surface [17]. Among these, the deposition of HA ($Ca_{10}(PO_4)_6(OH)_2$) on metallic implants from simulated body fluid (SBF) is one of the most widely investigated methods, due to their outstanding biological responses in a physiological environment [18,19]. Recently, a biomimetic process was employed for the formation of a HA layer on a heat treated Ti substrate in 1.5 times simulated body fluid (1.5 × SBF) with the addition of a recombinant collagen-like protein [20].

Actually, human bone is mainly composed of collagen and HA. In the past decade, using the biomimetic concept, many synthetic bone grafts, including apatite/collagen or apatite/gelatin composites, have been developed to realize compositional and structural analogy to natural bone as well as high biocompatibility in vitro and in vivo conditions [21–24]. However, these composite materials could not effectively replace the native bones due to their lower fracture toughness and mechanical properties.

Carbon nanotubes (CNTs) have an unique chemical structure, high electrical and thermal conductivity, high chemical stability, and a high surface-to-volume ratio [25,26]. CNTs show excellent mechanical properties such as a high Young's modulus (1.0–1.8 TPa), high tensile strength (60–150 GPa) and high elongation at break (10–30%). Moreover, the chemical modification of their surface is relatively easy and so the functionalization of CNTs with biomolecules has become an attractive topic in biomedical applications such as biosensors and drug delivery [27,28]. These

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outstanding properties make CNTs extremely attractive for use as reinforcement fillers with excellent biocompatibility in the development of nanohybrids for bone grafting [29–35].

In this study, one major focus was the natural bone-like biomimetic modification of the titanium surface using CNTs, gelatin, and HA. Herein, a natural bone-like surface could be created by mimicking the collagen fibrils containing the HA minerals in natural bone. For this purpose, carboxyl group-functionalized CNTs were covalently immobilized with the help of APTES (3-aminopropyltriethoxysilane) linkages on the titanium surface in the form of a monolayer, followed by the chemically covalent covering of the CNT fiber surfaces with gelatin molecules and the biomimetic deposition of HA crystals in SBF solution. The chemical immobilization of CNTs on the titanium surface and the chemical hybridization of gelatin and hydroxyl apatite on the surface of the CNT fibers might be a reasonable strategy to create a new natural bone-like surface with high fracture toughness, improved initial response of cells, and high ability to generate new bony tissue at the interface between the implanted materials and surrounding tissue.

2. Experimental

2.1. Materials and characterizations

Ti discs purchased from Seoul Titanium Co., Korea, having a diameter of 11 mm and thickness of 0.8 mm, were used as the experimental substrates. MWCNTs (>95%, 15–20 nm outer diameter, 10–20 μm length) were purchased from EMP (EM-Power Co., LTD, Korea). Gelatin (Gel, Mw = 2.0×10^4–2.5×10^4), 3-aminopropyltriethoxysilane (APTES), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) were purchased from Sigma–Aldrich (USA). All supplementary chemicals were of analytical grade and used without further purification.

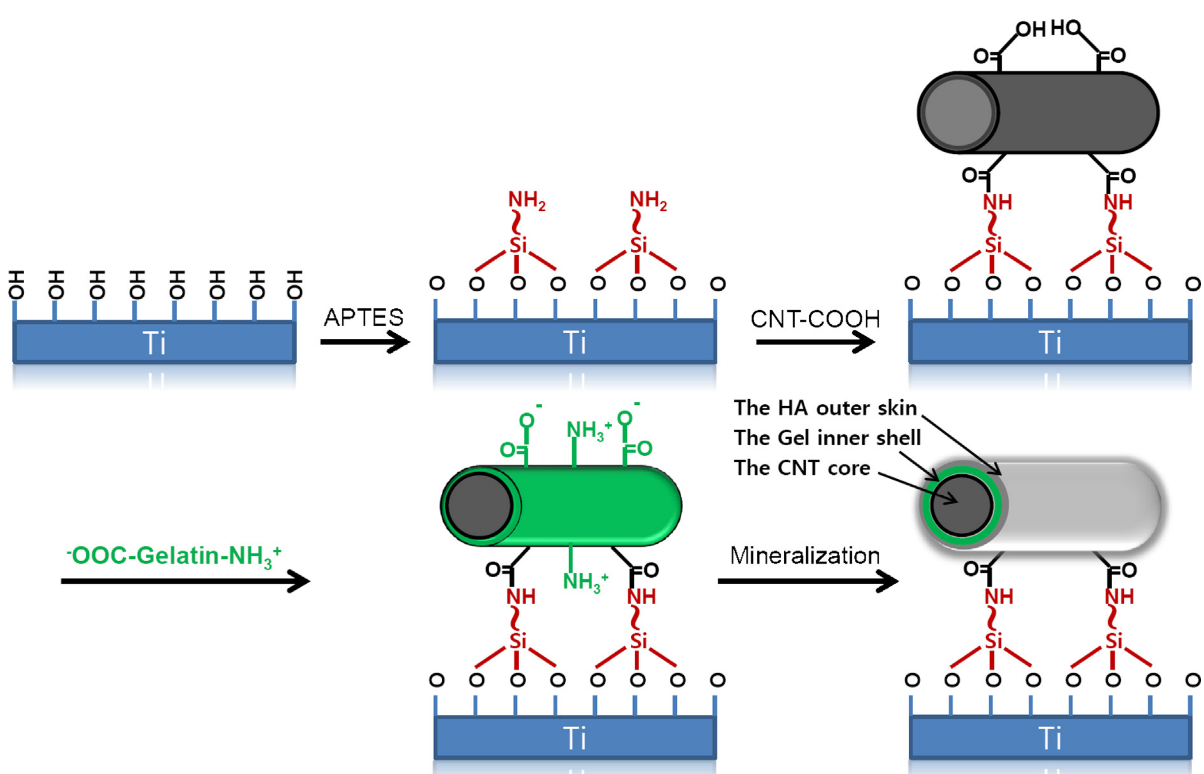
Scanning electron microscopic images (SEM) of the Ti substrates were obtained using an FE-SEM (MIRA II LMH, TESCAN, CZ) microscope. The specimens were sputter-coated with a thin layer of gold prior to examination. The wettability change for every coating step was followed by a contact angle instrument (Phoenix 300, Surface Electro Optics Co. Ltd, and Korea) at 25 °C and 30% humidity. Total five specimens for each step were tested and then the contact angle values were averaged. As a qualitative analysis, X-ray photoelectron spectroscopy (XPS) was conducted using a VG Microtech ESCA 2000 system with a monochromatized aluminum K_α anode (300 V, 27 mA). The hydroxyl apatite phase and composition of the sample surface were detected by X-ray diffraction (XRD, Rigaku).

2.2. Functionalization of carbon nanotubes

Carboxyl group functionalized multiwalled carbon nanotubes (MWCNT – COOH = CNTs) were prepared by the acid oxidative method. In a typical experiment, 2 g of CNTs were added to 100 mL of a 1:1 concentrated H_2SO_4/HNO_3 aqueous solution and the mixture refluxed at 80 °C for 2 days. After the reaction, the mixture was diluted using 100 mL of distilled water and then filtered through a 0.4 μm Millipore polycarbonate filter membrane. The resulting CNT powders were continuously washed using distilled water until the pH of the filtrates became 7. The CNTs were dried under vacuum for 24 h at 50 °C.

2.3. Pretreatment of Ti substrate

After ultrasonic cleaning in deionized water, acetone and ethanol, the native oxide layers of the Ti substrates were removed by soaking in 5 M NaOH solutions for 24 h. After neutralizing them in 2N HCl solution for 1 min, cleaning in distilled water, and drying, the Ti substrates were heat-treated at 600 °C for 2 h in a furnace to



Scheme 1. Overview of the procedure employed to create the CNT-core surrounded by gelatin and HA double-shells on the Ti implant surface.