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Synthesis of bio-functionalized three-dimensional titania nanofibrous structures using femtosecond laser ablation

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ABSTRACT

The primary objective of current tissue regeneration research is to synthesize nano-based platforms that can induce guided, controlled, and rapid healing. Titanium nanotubes have been extensively considered as a new biomaterial for biosensors, implants, cell growth, tissue engineering, and drug delivery systems. However, due to their one-dimensional structure and chemical inertness, cell adhesion to nanotubes is poor. Therefore, further surface modification is required to enhance nanotube–cell interaction. Although there have been a considerable number of studies on growing titanium nanotubes, synthesizing a three-dimensional (3-D) nano-architecture which can act as a growth support platform for bone and stem cells has not been reported so far. Therefore, we present a novel technique to synthesize and grow 3-D titania interwoven nanofibrous structures on a titanium substrate using femtosecond laser irradiation under ambient conditions. This surface architecture incorporate the functions of 3-D nano-scaled topography and modified chemical properties to improve osseointegration while at the same time leaving space to deliver other functional agents. The results indicate that laser pulse repetition can control the density and pore size of engineered nanofibrous structures. In vitro experiments reveal that the titania nanofibrous architecture possesses excellent bioactivity and can induce rapid, uniform, and controllable bone-like apatite precipitation once immersed in simulated body fluid (SBF). This approach to synthesizing 3-D titania nanofibrous structures suggests considerable promise for the promotion of Ti interfacial properties to develop new functional biomaterials for various biomedical applications.

1. Introduction

Nanofiber and nanotube architectures with high surface to volume ratios show functional and unique properties compared with those of their bulk counterparts. In particular, titania nanofiber and nanotube structures are of a great interest due to their proven biocompatibility, thermal stability, and corrosion resistance. They can be used for a number of applications, such as gas sensors, solar cells, implant surface modifications, tissue engineering, implantable drug delivery systems, and other medical devices [1–3]. Titania nanotube films have been widely recognized as growth support substrates for bone and stem cells, for the prevention of bacterial adhesion, and for enhancing blood clotting to control hemorrhaging. Recent in vivo and in vitro studies have demonstrated that surfaces comprised of nanotube platforms exhibit additional biological effects by integrating the oxide and apatite nanocrystals and also by improving cell–material interactions [1,4–6].

Titania nanotubes have been synthesized by several techniques, such as anodization [7], template-based synthesis [8], sol–gel transformation [9], and hydrothermal synthesis [10]. Among these, anodization continues to excite interest due to the simplicity of material preparation, as well as the greater control over the synthesis process in comparison with other methods. However, a multiple step process is required and material preparation is comparatively complex owing to the long process time and high temperature [11]. Also, the as-anodized nanotubes are amorphous and a high temperature annealing step is required to form the crystalline phase [12]. Furthermore, previous research has clearly indicated that additional surface modification of titania nanotubes is necessary to further improve their biocompatibility [1]. With respect to tissue regeneration, three-dimensional (3-D) porous structures would be more promising for scaffold systems than one-dimensional nanotubes, owing to their porous and interwoven structure. The merits of a surface comprised of interwoven ultrafine nanofibrous structures would be high porosity, a variable pore size distribution, a high surface to volume ratio and, most importantly, morphological similarity to natural extracellular matrix (ECM) [13].

The goal of our present work is to introduce a single step technique to synthesize titania nanofibrous structures using femtosecond laser ablation under ambient conditions. To the best of the

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authors' knowledge up to now there have been no reports on the synthesis of 3-D titania nanofibrous structures using femtosecond laser ablation. The 3-D titania nanofibrous structures would improve titanium surface properties for a wide range of biomedical applications. Under high repetition femtosecond laser irradiation materials attain high temperatures and pressures and cool down in an extremely short time, thus generating material states which cannot be synthesized using low repletion long pulse lasers. We have also determined that the nanostructure pore size and nanofiber density on the surface could be controlled through adjustment of the laser parameters, such as laser pulse repetition and dwell time. The morphology, phase analyses, and interfacial properties of the synthesized nanofibrous structures have been characterized by scanning electron microscopy (SEM) followed by energy dispersive X-ray spectroscopy (EDS), transmission electron microscopy (TEM), X-ray diffraction (XRD) analysis, and contact angle measurement. The bone-like apatite-inducing ability of Ti surfaces with different morphology has been evaluated using simulated body fluid (SBF).

2. Experimental section

2.1. Laser processing and generation of nanofibrous structures

A titania nanofibrous structure has been formed on Ti samples using single point femtosecond laser irradiation under ambient conditions. Substrate samples $10 \times 10 \times 2$ mm were cut from grade 2 (ASTM B265) pure Ti sheet using a diamond saw with oil lubrication. The samples were then ground progressively using 180, 320, 400, 600, and 1200 grit silicate-carbon papers to remove macro-level surface defects and contaminants. Once ground they were ultrasonically cleaned in distilled water and dried in a desiccator. Lastly, specimens were irradiated by laser beam at laser pulse repetitions of 4, 8, and 12 MHz to generate nanofibrous structures of different densities and with different pore sizes. The laser pulse width, power and irradiation dwell time were 214 fs, 15 W, and 5 ms, respectively. The laser source used in this experiment was a 1040 nm wavelength direct diode pumped, Yb doped fiber amplified ultrafast system. Due to the solid-state operation and high spatial mode quality of fiber lasers our system produced low noise performance. In addition, the laser parameters, including laser repetition rate, pulse width, and beam power, are computer controlled, allowing accurate interaction in the performed experiments. Fig. 1 is the schematic diagram of the experimental setup and procedure.

Laser irradiation of a target creates a heated region, which causes vaporization, leading to formation of plasma plume. As the plume expands outwards its temperature and pressure decrease, resulting in condensation, which leads to nucleation. At laser pulse repetition rates higher than the nanoparticle formation threshold successive laser pulses irradiating the target surface maintain a continuous flow of the vapor plume, which consequently increases the nucleus density. The large number of nuclei leads to the growth of nanoparticles rather than micro-scale droplets, which will aggregate into interwoven nanofibrous structures after further collisions.

2.2. Sample soaking in SBF for in vitro assessment

The effect of surface morphology on apatite-inducing ability was evaluated by soaking the samples in SBF with ionic concentrations nearly equal to human blood plasma (Table 1). A modified simulated body fluid (m-SBF) was prepared by dissolving the following reagents in sequence in distilled water: NaCl, $NaHCO_3$, Na_2CO_3, KCl, $K_2HPO_4{\cdot}3H_2O$, $MgCl_2{\cdot}6H_2O$, $CaCl_2$, and Na_2SO_4. The solution was buffered to pH 7.40 with HEPES and 1 M NaOH at 37 °C [14]. Each Ti sample was then placed in a sterilized polyethylene container with 30 ml SBF and kept in an incubator at 37 °C for 1 or 3 days. After exposure the samples were removed and washed thoroughly with distilled water and dried in a desiccator for further characterization.

2.3. Surface characterization

The morphology of the nanofibrous structures before and after SBF soaking was characterized using SEM followed by EDS analysis. Nanoparticle aggregation and the size of the nanofibers were analyzed by TEM. In order to separate the nanostructures from the substrate samples were immersed in isopropanol solution and ultrasound vibration was applied. Then a drop of the dispersed nanofiber solution was placed on a copper mesh and allowed to dry in a desiccator.

Phase analysis of the synthesized structures was performed using XRD. The X-ray source was a CuK_α rotating anode generator with a parallel focused beam and three-circle diffractometer with a two-dimensional detector. The average wavelength of the X-rays was 1.54184 Å. Phi scans with widths of 60° were done with the detector at four different swing angles for each sample in order to obtain a profile with a 2θ range of 10.5–104°.

2.4. Contact angle measurement

The wetting properties of the nanofibrous structure were studied by dynamic contact angle measurements. Samples were fixed and a droplet of distilled water was applied to the surface. Images of each drop on the surfaces were recorded using a digital microscope. The contact angles were determined from the images using axisymetric drop shape analysis (ADSA-NA) methodology [15]. The mean value of the contact angles was calculated from five individual measurements taken at different locations on the substrates.

3. Results and discussion

3.1. The structure of the nanofibrous architecture

The structure of the nanofibrous architecture is influenced by various laser parameters, such as laser fluence, laser pulse repetition and laser pulse dwell time. In this study we have investigated the effect of laser pulse repetition on porosity and size of the synthesized nanofibers. Fig. 2 shows SEM micrographs of the nanofibrous structure generated on the Ti4 surface at a pulse repetition rate of 4 MHz. A close-up view of the structure shows that it consisted of self-assembled closed rings and bridges in which nanoparticles are fused together. The pores are interconnected, with sizes of 900–1000 nm. Additional experiments have been performed with different laser repetition rates of 8 and 12 MHz (Fig. 3). The pore sizes range from approximately 700 to 800 nm for the nanofibrous structures synthesized on Ti8, while they are about 650–750 nm for those synthesized on the surface of Ti12. In TEM micrographs of a single nanofiber one can observe a high degree of nanoparticle aggregation (Fig. 4). The nanoparticles are aggregated together in a semi-solid state rather than loosely agglomerated. Therefore, the bonds between the particles themselves and with the Ti substrate are assumed to be strong. The diameter of the nanofibers is approximately 16–20 nm for Ti12 and increases as the repetition rate is reduced to 4 MHz. These results indicate that a reduction in laser pulse repetition rate leads to an increase in the density of the nanofibrous structures as well as in the size of nanoparticles. This is due to the fact that at constant laser power and laser spot size pulse energy drops off with an