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ORIGINAL ARTICLE

Enhanced osteointegration of medical titanium implant with surface modifications in micro/nanoscale structures

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Summary Biomimetic design and substrate-based surface modification of medical implants will help to improve the integration of tissue to its material interfaces. Surface energy, composition, roughness, and topography all influence the biological responses of the implants, such as protein adsorption and cell adhesion, proliferation and differentiation. In the current study, different surface structures of Ti implants were constructed using facile surface techniques to create various micro-, nano-, and nano/micro composite scale topography. We have fabricated three types of hierarchical structures of TiO_2 coating on Ti implants, including nanotube

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structure, nano sponge-like structure, and nano/micro nest-like structure. The osteointegration and biomechanical performance of the coated Ti screws were evaluated by histology and removal of torque force test *in vivo*. We found that the nano/micro nest-like and nanotube structured surface possessed better osteointegration ability. It indicated that the alkaline hydrothermally treated Ti substrate was the best for bone-implant integration in terms of all *in vitro* and *in vivo* testing parameters. The alkaline hydrothermally treated surface displayed a hydrophilic (contact angle value 5.92 $\pm$ 1.2), higher roughness (Ra value 911.3 $\pm$ 33.8 nm), higher specific surface area (8.26 $\pm$ 1.051 m^2/g), and greater apatite inductivity. The electrochemical surface modification may become a powerful approach to enhance metal implant to bone integration in orthopaedic applications.

Introduction

Ti-based materials have been widely used in orthopaedics and dental surgery as implants because of their strong mechanical properties and good chemical stability and biocompatibility [1]. To enhance osteogenetic differentiation and osteointegration of implants, various treatments have been developed to modify the surface chemistry, physical properties, and topography; approaches include machining/micromachining, sandblasting, acid etching, electropolishing, anodic oxidation, and plasma spraying [2—5]. These techniques mainly alter the surface parameters, leading to enhancement of cell attachment to the implant surfaces. In the assays, cells grow and integrate better on the modified surfaces by producing more extracellular matrix components, including cell adhesive proteins or type I collagen and fibronectin [6,7]. However, the results from *in vitro* studies may be different from the *in vivo* situation. Osteointegration is the direct anchorage of an implant by the formation of bony tissues around the implant without the growth of fibrous tissues at the bone—implant interfaces [8], which is influenced by a wide range of factors including anatomical location, implant size and design, surgical procedure, loading effects, biological fluids, age, sex, and particularly, the implant surface characteristics [9]. Torque removal force has been used as a biomechanical measure of anchorage or osteointegration in which the greater force required to remove implants may be interpreted as an increase in the strength of osteointegration.

Surface composition, topography, wettability, roughness, and surface electrical charge are key parameters in determining implant—tissue interaction and osteointegration [10—12]. Roughness of the implant surfaces usually results in cellular adherence through guiding the cytoskeletal assembly and membrane receptor organization and accelerating the adsorption of fibronectin and albumin [13,14]. A positive relationship has been found between bone-to-implant contact and implant surface roughness [15]. Sandblasted and acid-etched surface implants are nowadays commercially available as dental implants with microscale average roughness [16]. Gittens et al [17] introduced nanoscale structures with micro/submicroscale roughness without chemical composition and contact angles modification, and improved the osteoblast differentiation and local growth factors production. Zinger et al [18] reported that with surface modification of Ti material with hierarchical surface roughness, osteoblasts responded differently towards nano-, micro-, or nano/micro topography. The pattern, size, and distribution of peaks and valleys that compose the surface roughness are also important factors influencing osteointegration.

The optimal surface roughness for metal implant osteointegration has not yet been well defined. A thick surface oxide layer is desirable because of its ability for hydrocarbon adsorption [9]. Medical devices might also benefit from hydrophilic surface treatment to reduce interfacial inflammatory responses and promote bony ingrowth [1]. In this study, different Ti implant surface structures were constructed using facile surface techniques to create various micro-, nano-, and nano/micro composite scale topographies, including nanotubular structure, nano sponge-like structure, and nano/micro nest-like structure. The surface was characterized by various physicochemical methods, and the bioactivity and osteointegration were investigated through *in vitro* and *in vivo* evaluations.

Materials and methods

Preparation of coatings

Ti foils (Baoji Titanium Industry Co. Ltd., China) of 0.1-mm in thickness and 99.5% purity were used. Prior to the different treatments, the Ti sheets (10 mm $\times$ 10 mm) were degreased in an ultrasonic bath in acetone, anhydrous ethanol, and deionised (DI) water successively, followed by rinsing with DI water, and drying in air.

TiO_2 nanotubular structure

The electrochemical anodisation of the Ti sheet was carried out by using a DC voltage source. Anodic films of TiO_2 nanotubes were grown by potentiostatic anodisation at 20 V using a Pt sheet as a counter electrode in 0.5% hydrofluoric acid solution under stirring conditions at room temperature. The samples were sufficiently rinsed with DI water after anodisation, and calcined at 450°C for 2 hours [19].

TiO_2 sponge-like structure

All the preparation conditions for TiO_2 sponge-like structure were the same as for the above experiments, except the anodising potential was set at 50 V [20].