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Nanotextured titanium surfaces for enhancing skin growth on transcutaneous osseointegrated devices

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ABSTRACT

A major problem with transcutaneous osseointegrated implants is infection, mainly due to improper closure of the implant–skin interface. Therefore, the design of transcutaneous osseointegrated devices that better promote skin growth around these exit sites needs to be examined and, if successful, would clearly limit infection. Due to the success already demonstrated for orthopedic implants, developing surfaces with biologically inspired nanometer features is a design criterion that needs to be investigated for transcutaneous devices. This study therefore examined the influence of nanotextured titanium (Ti) created through electron beam evaporation and anodization on keratinocyte (skin-forming cell) function. Electron beam evaporation created Ti surfaces with nanometer features while anodization created Ti surfaces with nanotubes. Conventional Ti surfaces were largely micron rough, with few nanometer surface features. Results revealed increased keratinocyte adhesion in addition to increased keratinocyte spreading and differences in keratinocyte filopodia extension on the nanotextured Ti surfaces prepared by either electron beam evaporation or anodization compared to their conventional, unmodified counterparts after 4 h. Results further revealed increased keratinocyte proliferation and cell spreading over 3 and 5 days only on the nanorough Ti surfaces prepared by electron beam evaporation compared to both the anodized nanotubular and unmodified Ti surfaces. Therefore, the results from this in vitro study provided the first evidence that nano-modification techniques should be further researched as a means to possibly improve skin growth, thereby improving transcutaneous osseointegrated orthopedic implant longevity.

1. Introduction

Percutaneous implants encompass any device that penetrates the skin and is exposed to the outside environment, including catheters for dialysis, blood tubes for ventricular assist systems, external bone fixation devices and transcutaneous osseointegrated devices (TODs) for limb prostheses. All percutaneous devices suffer from the serious problem of infection caused by bacteria that eventually lead to the breakdown of the skin–device interface [1–5]. This breach in the physical barrier is due to the current inability of creating biomaterials that can create a tight seal between the skin and the implant device. Most percutaneous devices are designed to function for only a short period of time due to prominent infection rates. However, TODs for limb restoration are implanted for the long term, thus requiring a strong need to circumvent problems of poor skin growth around the implant device, while simultaneously limiting infection.

A lack of successful skin integration around the TOD exit sites leads to reduced fixation strength and failure to protect against bacterial invasion [6,7]. As bacteria colonize either the implant surface or adjacent damaged tissue sites, biomaterial exit sites become the gateway to infection [8–18], possibly leading to bacteria spreading internally and causing osteomyelitis [18,19]. According to previous studies, the occurrence of osteomyelitis after insertion of an external fixator is anywhere between 0% and 4% [8–19]. In addition to osteomyelitis, infection leads to bone implant loosening [14–17] and fracture malunion or nonunion of TODs.

Current methods are aimed at preventing bacterial infection rather than repairing the lack of skin growth around the TOD exit site. Such methods include altering surgical techniques, modifying the implant design and coating the device with a new material. Coating approaches to improve the anti-bacterial properties of TOD include using fibroblast growth factor-2-embedded apatite composites [20] as well as silver [21–24] and hydroxyapatite coated implants [14,25–31]. For example, titanium screws coated with fibroblast growth factor-2-embedded apatite composites showed a 50% decrease in infection compared to those without

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the composite layer [20]. Similarly, one study revealed that 62% of silver coated pins became infected compared to 84% of uncoated pins [22]. Clearly, these coatings possess properties that reduce the presence of bacteria compared to their uncoated counterparts, yet the presence of infection is still high (due to delamination concerns), indicating there is a need for an alternate means to decrease infection rates. An alternative strategy is to create implant surface properties that can promote skin growth around the exit site, which would not only provide device stability but would also provide a permanent barrier for bacteria invasion and, thus, infection.

To achieve secure and stable device integration between the skin and the metal implant surface, developing surface properties that would promote and support keratinocyte (skin-forming cell) adhesion and proliferation, and consequently decrease infection, would be advantageous. Along this line, natural tissues, including skin, are composed of numerous nanostructures involved in cellular interactions and processes [32]. Therefore, it may be ideal to design implant surfaces that incorporate such nanometer features to allow for optimal interactions with proteins and subsequently cells [33,34]. It has been shown that biologically inspired nanometer surfaces possess increased surface roughness, surface energy and hydrophilicity, which promote cellular responses (such as adhesion, proliferation, differentiation and migration) and eventually tissue regeneration [35–40] by better mediating initial protein adsorption and bioactivity (such as from fibronectin and vitronectin [35,41–43]). Specifically, in the orthopedic field, research has clearly demonstrated that nanostructured surfaces promote bone growth more than current conventional (non-nanostructured) surfaces [44–59], particularly on Ti and Ti alloys [60–62]. In addition to promoting cell attachment and growth, these nanometer features have been shown to decrease bacterial attachment [63–65], thus potentially providing a solution to the three major issues associated with TODs (poor skin and bone growth and infection).

The methods used to fabricate these nanometer features on metals include chemical etching (such as via HNO_3) and powder metallurgy techniques [45]. Powder metallurgy techniques involve compacting metal nanometer powders, such as Ti, Ti6Al4V and CoCrMo, into circular discs using a hydraulic press; such materials do not possess the mechanical strength necessary for implantation. In addition, to ensure that cells are only responding to changes in topography and not other parameters, it is also important to choose nano-modification techniques that create surfaces varying only in roughness. Therefore, in this in vitro study, the effects of nanotextured Ti surfaces on the behavior of keratinocytes were studied as a means of initially determining ways to improve the interface between the skin and the implant for TODs in limb restoration. In particular, nanotubular and nanorough Ti surfaces were prepared in this study by anodization and electron beam evaporation techniques, respectively.

2. Materials and methods

2.1. Substrate preparation

Nanotubular and nanorough titanium (Ti) substrates (10 × 10 × 1 mm) were prepared using 99.2% pure Ti foils (Alfa Aesar, Ward Hill, MA, USA). The foils were ultrasonically cleaned with diluted formulated cleaning solution (Branson, Dabury, CT, USA) for 20 min followed by sonication in acetone, 70% ethanol and deionized water (DI) for 10 min and then were dried in an oven at 40 °C for 15 min.

Nanotubular Ti substrates were fabricated by first soaking Ti in a dilute acidic mixture of nitric acid (HNO_3) and hydrofluoric acid (HF) for 5 min in order to remove the thin oxide layer that spontaneously forms on Ti while in air. Titania nanotube arrays were then formed on the Ti surface by anodization (Fig. 1). Anodization is an

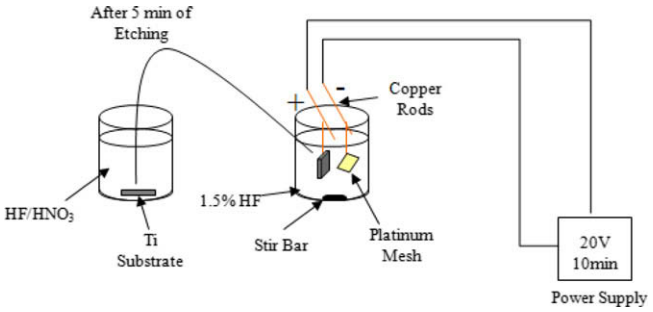

Fig. 1. Representation of the anodization process (an electrolytic passivation process used to increase the thickness of the natural oxide layer on metal surfaces) used to create the nanotubular Ti surfaces.

electrolytic passivation process used to increase the thickness of the natural oxide layer on metal surfaces, in this case Ti. This process was conducted with a DC-powered electrochemical cell that had a two-electrode configuration: a platinum mesh, which served as the cathode, and Ti, which served as the anode. The anodization process took place in 1.5 wt.% HF for 10 min at a constant voltage of 20 V [66]. These Ti substrates were rinsed with large amounts of DI immediately after anodization, air dried and sterilized under ultraviolet light for 3 h per substrate side prior to cell culture experiments.

Nanorough Ti substrates were fabricated using a Temescal Electron Beam Evaporator (Reston, VA, USA). Electron beam evaporation (Fig. 2) concentrates a large amount of heat produced by high-energy electron beam bombardment on the source material to be deposited, in this case 99.995% pure Ti pellets (Kamis, Mahopac Falls, NY, USA). The electron beam is generated by an electron gun that uses the thermionic emission of electrons produced by an incandescent filament. A magnet focuses and bends the electron trajectory so that the beam is accelerated towards a graphite crucible (Lesker, Clairton, PA, USA) containing the source material. As the beam rotates and hits the surface of the source material, heating and vaporization occur. The vapor flow then condenses onto the substrate surface located at the top of the vacuum chamber. In this study, Ti was deposited onto the Ti substrates at a rate of 3.5 Å s^{-1} and at a thickness of 500 nm. Following deposition, the Ti samples were rinsed thoroughly with DI and air dried.

The Ti foils whose surfaces were not altered by either of these methods served as the conventional, unmodified samples. Borosilicate glass coverslips (reference material) obtained from Fisher Scientific (Agawam, MA, USA) were degreased ultrasonically in acetone and 70% ethanol for 10 min each, etched in 1N NaOH for

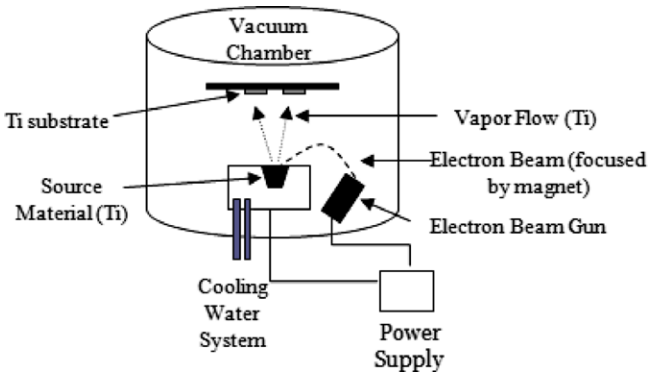

Fig. 2. Representation of the electron beam evaporation process (high electron beam bombardment used to deposit a material, in this case Ti, onto another material, also Ti here) used to create the nanorough Ti surfaces.

ID	Title	Pages
1444	Nanotextured titanium surfaces for enhancing skin growth on transcutaneous osseointegrated devices	11