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Amino acid conjugated self assembling molecules for enhancing surface wettability of fiber laser treated titanium surfaces

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ABSTRACT

Surface wetting properties of implants are one of the most critical parameter, which determine the interaction of proteins and cells with the implant surface. In this regards, acid etching and sand blasting are the mostly used methods at surface modification of Titanium (Ti) for enhanced surface wettability. Besides, these kinds of modifications may cause a conflict whether the surface wettability is influenced by the process related surface contaminations or by the surface roughness. In contrast, lasers might be an option for the alteration of surface wetting properties via supporting micro and/or nano surface topographies while preventing surface chemical contaminations. In this work, we focused on two steps of surface processing approaches of Ti surface: physical and chemical modifications. Herein, we hierarchically structured Ti surfaces by using microsecond modulated pulsed fiber laser. Subsequently, laser structured and non-structured Ti surfaces were further modified with novel histidine and leucine Amino Acid conjugated Self-Assembled Molecules (His[1]-SAMs[2] and Leu[3]-SAMs) to alter the surface wettability by introducing biologically hydrophilic and hydrophobic groups. Modification of Ti surfaces with His-SAMs and Leu-SAMs ended up with stable wetting properties when compared to non-modified surfaces after 7 days which may enhances the cell–surface interaction.

1. Introduction

Mechanical performance and chemical inertness of Ti and its alloys inside the body and especially their strong interaction with the osteoblast cells make them an ideal and indispensable material for the medical implantations. Therefore, Ti and its alloys are widely used in such as, orthopedic and dental medical implantations for decays [1–8].

On the other hand, although Ti and its alloys are one of the most preferred implant materials, their surfaces may be needed for further treatments in order to enhance the biocompatibility and osseointegration, if necessary [6,9,10]. As already known, cells adhere, spread and proliferate over the implant surfaces subsequent to the surface protein adsorption [4,11,12]. When a material gets in contact with the body fluids, protein adsorption occurs via the adsorption of water molecules [13]. In that circumstance, surface wettability gains extreme importance for an enhanced cell activity over the implant surface and the wetting properties of an implant can be controlled by modifying its surface chemistry and surface topography [4,7,11–22].

Introducing biocompatibility to the implant surfaces can be achieved by chemical and physical surface modifications. It has been reported that deposition of calcium phosphate (CaP) and hydroxyapatite (HA) based layers over materials surfaces enhance the osteoblast activity [11,16–18]. As shown by Thian et al., nano silicon substituted HA coating on glass substrates show enhanced hydrophilic behavior in comparison to pure HA layers which resulted with enhanced alkaline phosphatase, Collagen Type-I and osteocalcin expression [23]. Instead of inorganic/chemical and organic/chemical biocompatible surface modifications, metallic implant materials can be modified with silane group containing self-assembled molecules as interface molecule, such as: (3-Aminopropyl)triethoxysilane (APTES), since these molecules are capable to make covalent bonds with Ti and its alloys via surface oxygen groups [24,25]. Müller et al. indicated that the introducing collagen layer over oxidized Ti, Ti_6Al_4V and Cobalt via immobilization of amino-functionalized silane coupling agents resulted with

Abbreviations: His, histidine; SAMs, self assembling molecules; Leu, leucine.

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the enhancement of adhesion and proliferation of human immortalized osteoblast-like cells MG-63 [25]. Last but not the least, biochemical surface modification of materials is another approach, which is based on the deposition of organic components of bone extracellular matrix (ECM) proteins, such as Collagen Type-I [26]. As reported by Roehlecke et al., coating of collagen type-I protein over Ti_6Al_4V surfaces enhances the proliferation, spreading and attachment of primary osteoblast cells in comparison to the uncoated surfaces [19]. Including the biochemical and chemical modifications, physical methods such as increasing the surface roughness by introducing nano scale structures to the surface by using acid etching and/or sand blasting, enhance the surface wetting properties and resulted migration and anchoring of the osteoblast cells to the implant surfaces have been studied in the literature [7,13–15,18]. It was reported by Szmukler-Moncler et al. that increasing the surface roughness by using sandblasting and chemical etching processes increase the bone formation on Gr-4 Ti implants [14]. Similarly, Rosales-Leal et al. presented the enhanced MG-63 osteoblast-like cells activity on Ti surfaces, which were chemically etched and sandblasted with Al_2O_3 particles and representing hydrophilic nature [27]. Another study by Tran and Webster indicated that introducing Selenium (Se) nano particles over Ti implants at different densities increase the surface hydrophobicity. They concluded that Se nano particles trigger the absorption of osteoblast cell adhesive proteins while supporting the reduction of bacterial adhesion [13].

Although modification methods mentioned above exhibited enhanced cell activities over the implant surfaces, these type of modifications induce changes at the surface chemistry via chemical residues, which may cause a conflict if surface wettability is influenced by the surface chemistry or the surface topography [12,28]. In contrast, lasers can be used to enhance only the surface topography while not changing the surface chemistry [7,12,28–33]. It was reported that structuring of aluminum oxide substrates by laser sources, which is one of the preferred material especially for orthopedic implants, has no influence on the surface chemistry of the material [12,28–31]. Additionally, using an assist/shielding gas during the laser surface structuring can be used to protect the original surface chemistry via blocking the further surface oxidation and nitriding [7,32]. As reported by Chikarakara et al., CO_2 laser processed Ti_6Al_4V substrates under argon shielding gas atmosphere shows enhanced cell growth and proliferation in comparison to non-treated substrates [33]. Besides, introducing ultra-thin layers of chemical groups over morphologically enhanced implant surface shows enhanced cell activity since the deposited layers cover all the surface features completely and therefore rules out the conflict of the question “surface chemistry or surface topography” [12,28].

In this respect, we focused on enhancing surface wettability of commercially available Grade-2 Ti substrates through easy, cheap but simple and efficient topographical and chemical modifications. Topographical modification was performed by using microsecond pulsed fiber laser for the generation of hierarchical micro and nano surface structures inspired from the natural hierarchical structure of bone [21] while chemical modifications were performed by using novel amino acid (histidine and leucine) conjugated SAMs via altering the surface wettability in order to develop more biocompatible, reproducible, predictable, stable and well defined implant surfaces.

2. Experimental

2.1. Pre-preparation of Ti surfaces and cleaning

Ti substrates (15 mm diameter) were cut from commercially pure Grade-2 Ti plates by using fiber laser cutting system (JK400F-GSI laser, UK), which operates at 1080 nm of wavelength and can be modulated up to 50 kHz of frequency. Laser-cut substrates were cleaned with absolute ethanol and sonicated in ultrasonic bath for 30 minutes with deionized H_2O before laser structuring process. Ti samples were structured by using the same laser in order to produce hierarchical surface structures in micro and nano-scale by using the ablation mechanism of laser material processing [34]. Schematic illustration of laser surface processing is shown in Scheme 1a. In order to find the required parameter that supports hierarchical surface structures, samples were scanned in *x*- and *y*-axis under stationary laser beam with a speed of 50.8 mm/s with different laser parameters. Step size of *y*-axis was fixed to 90 μm. Argon was used as shielding gas with a new design 6 mm output nozzle tip during the surface structuring process. Substrates were further cleaned with UV-Ozone cleaner (Jelight Company, Inc, USA) for 45 min prior to the chemical modification with amino acid conjugated molecules.

2.2. Synthesis of histidine and leucine conjugated self assembled molecules

Novel 2-amino-3-(1H-imidazol-4-yl)-N-(3-(trimethoxysilyl) propyl)propanamide [His-Si$(OCH_3)_3$] and 2-amino-4-methyl-N-(3-(trimethoxysilyl)propyl)pentanamide [Leu-Si$(OCH_3)_3$] were synthesized using our previous procedure [35] (Scheme 2) via N-acylbenzotriazole in order to introduce self-assembling properties to the selected amino acids. Preparation procedures and Nuclear Magnetic Resonance (NMR) data for the compounds were given in supporting information (S1-S3).

2.3. Surface chemical modification

Silane based molecules with methoxy and ethoxy functionalities are able to form self-assembled monolayers on Ti surfaces, therefore, histidine (His) and leucine (Leu) amino acids conjugated SAMs were herein synthesized, characterized in details and their solutions prepared at a concentration of 5 mM in absolute ethanol were subsequently used for chemical modification of Ti substrate surfaces [24,36]. Ti substrates were immersed in SAM solution for 2 h in order to functionalize the Ti surfaces. Following the functionalization process, substrates were rinsed with absolute ethanol for 3 times and dried under N_2 stream. Afterwards, Ti substrates were baked in oven for 15 min at 80 °C for the stabilization of SAMs. Chemical structure of modified Ti surfaces with His-SAMs and Leu-SAMs are shown in Scheme 1b.

2.4. Surface characterization

Scanning Electron Microscopy (SEM) (XL30 ESEM, Philips Corporation, Netherland) was used to analyze the surface topography and morphology of the hierarchically structured Ti substrates by fiber laser treatment in high resolution and magnification. Working Distance (WD) of the SEM was fixed for all characterizations and acceleration voltage was varied between 5 kV and 10 kV as required.

Roughness properties of the Ti surfaces were examined by using interferometric whitelight surface profiler (NewView 7200, Zygo Corporation, USA) and Atomic Force Microscope (AFM) (Ambios-Quesant Q-Scope Universal SPM, USA) operating at tapping mode. Roughness values were calculated by calculating the mean value of 3 randomly selected areas.

Surface chemistries of Ti substrates before and after laser and His-SAM and Leu-SAM modifications were analyzed with X-Ray Photoelectron Spectroscopy (XPS), which uses Al-Kα source gun (K-Alpha+ X-ray Photoelectron Spectrometer, Thermo Scientific, USA).

Static water contact angles measurements were performed at room temperature by using sessile drop method with contact angle equipment using ultrapure water (CAM-100, KSV Instruments,