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Macrophage polarization, inflammatory signaling, and NF-κB activation in response to chemically modified titanium surfaces

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ABSTRACT

Functionalization of titanium devices with various bioactive molecules enhances many of their properties as implants, including biocompatibility, which is typically assessed by macrophage activation and inflammation. However, functionalization requires prior introduction of reactive groups, to which bioactive agents can then be grafted. Thus, we investigated the inflammatory properties of titanium pretreated with NaOH, titanium pretreated with NaOH and then with 3-aminopropyl triethoxysilane, and titanium pretreated with dopamine. Inflammation, macrophage polarization, and activation of NF-κB signaling were assessed by real-time PCR and western blotting. The data demonstrate that silanized titanium is the least inflammatory, and promotes macrophage M2 polarization with modest engagement of the NF-κB signaling pathway. Importantly, silanization introduces a reactive amino group, providing more opportunities for further functionalization.

1. Introduction

Titanium and titanium alloys are currently the most widely used orthopedic implants due to excellent mechanical, physical, and chemical properties, biocompatibility [1]. However, native titanium is bio-inert, does not bond directly to surrounding bone tissue, and is incorporated into bone very slowly. Thus, Ti is often biofunctionalized to acquire novel properties [2–4]. For example, functionalization with antibiotics reduces bacterial adhesion and biofilm formation, while covalent addition of bioactive molecules promotes adhesion and proliferation of bone marrow mesenchymal stem cells and induces osseointegration [5,6]. Of note, bioactive components are sometimes deposited in layers [7–9].

In any case, biofunctionalization requires prior introduction of reactive groups such as oxhydryl (—OH), amino (—NH2), and carboxyl moieties (—COOH) [10], to which bioactive molecules can then be grafted. Accordingly, titanium is often pretreated with various chemical agents that also often alter surface topography and chemistry. For example, pretreatment with acid or alkali may alter surface morphology, chemical composition, roughness, and hydrophilicity, and thus affect biological properties independently of the bioactive molecules that are subsequently added. Indeed, rough surfaces may promote cell adhesion, proliferation, and contact osteogenesis [11]. Similarly, nanostructured surfaces may reduce macrophage recruitment and activation [12,13], and enhance osseointegration as well [14]. Hydrophilic surfaces may also inhibit expression of pro-inflammatory cytokines and chemokines such as TNF-α, IL-1α, IL-1β, and CCL-2 [15].

Macrophage-induced inflammation is an important index of biocompatibility, since macrophages are the primary drivers of the immune response to biomaterials [16]. Indeed, inflammation critically affects implant performance *in vivo*, including in osseointegration [14,17–19]. Hence, the inflammatory properties of titanium coated with reactive groups prior to functionalization should also be assessed. Accordingly, we investigated the inflammatory properties of titanium pretreated with NaOH, titanium pretreated with NaOH and silanized with 3-aminopropyl triethoxysilane, and titanium pretreated with dopamine. The data provide better understanding of the biological properties of titanium pretreated in different ways with various agents, and highlight silanization as a good approach to introduce a reactive group and thereby enable further functionalization.

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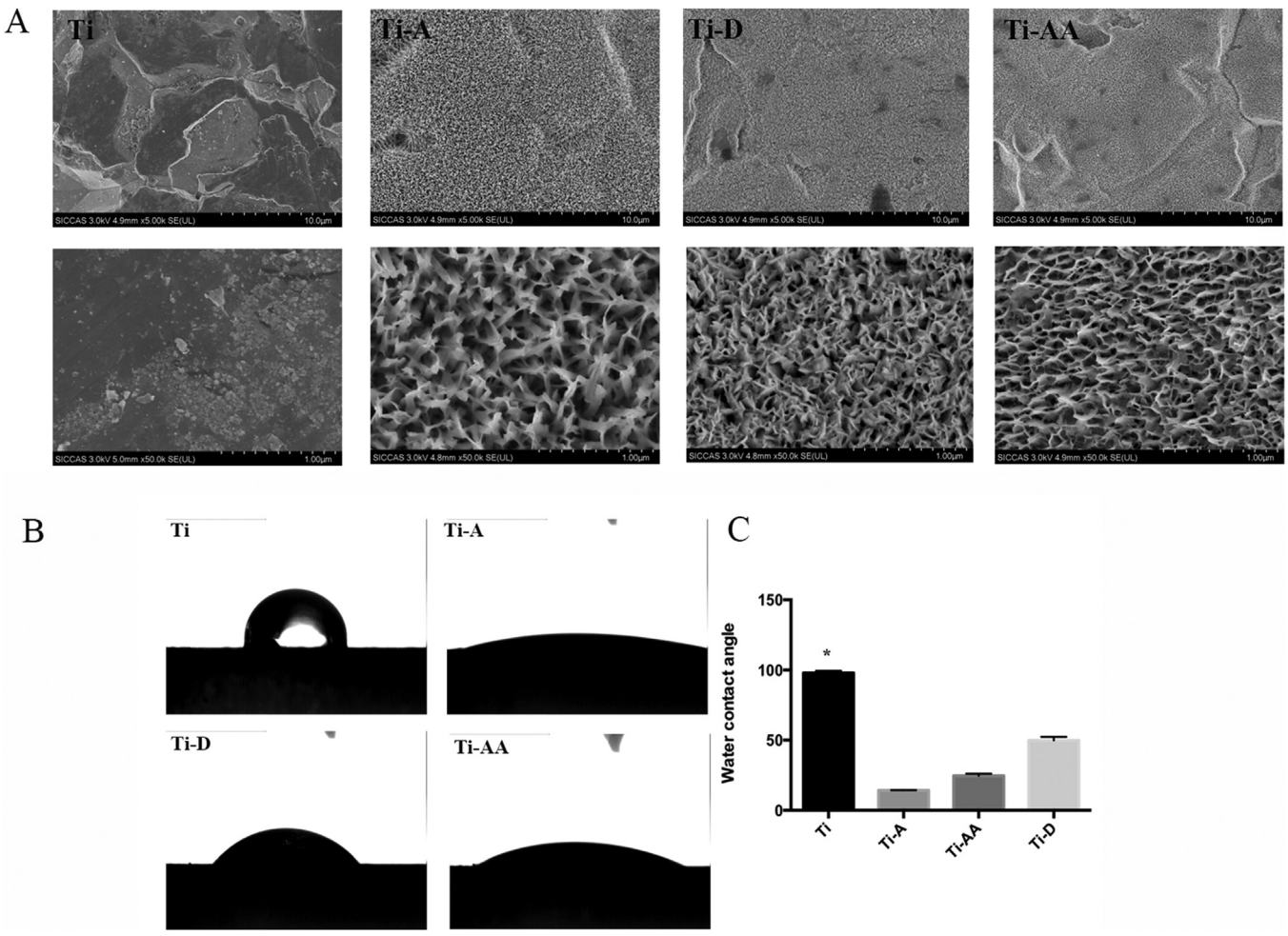

Fig. 1. (a) Surface characterization and (b, c) water-contact angle of native Ti, Ti derivatized with 5 M NaOH to introduce oxhydryls, Ti coated with dopamine, and Ti pretreated with NaOH and silanized. *, $p < 0.05$.

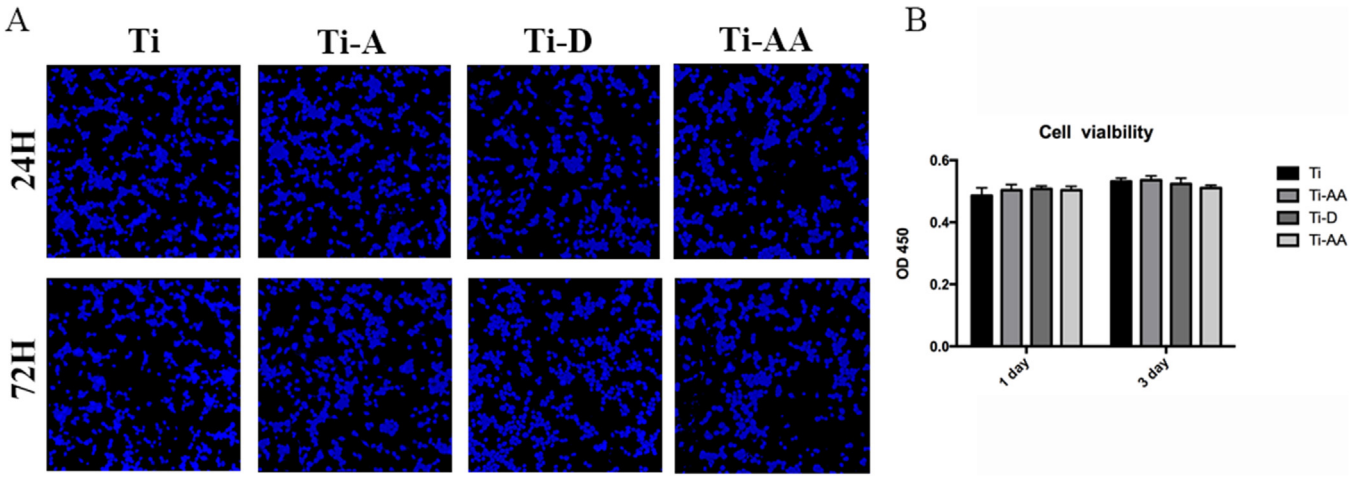

Fig. 2. Effects of different Ti surface modifications on THP-1 macrophages. (a) DAPI staining and (b) cell viability of macrophages seeded for 1 and 3 days on native and derivatized Ti (Native titanium was pretreated with 5 M NaOH to introduce oxhydryls denote as Ti-A. Native titanium were pretreated with NaOH and silanized with 3-aminopropyl triethoxysilane to introduce amino groups Ti-AA).

2. Materials and methods

2.1. Materials

Ti6AL4V (titanium alloy), NaOH, Tris–HCl pH 8.6, 3-aminopropyl triethoxysilane, and dopamine were obtained from Sigma-Aldrich, along with 0.5% trypsin–EDTA, 4′,6-diamidino-2-phenylindole (DAPI) and secondary antibodies conjugated to Alexa Fluor 488. cDNA synthesis kits and SYBR Premix EX Taq real-time PCR kits were procured from TaKaRa, while Pierce™ BCA Protein Assay Kit was purchased from Thermo Fisher. RNA mini kits were obtained from Qiagen, while alpha minimum essential medium (a-MEM)