Acta Biomaterialia 6 (2010) 3242–3255

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Stability of antibacterial self-assembled monolayers on hydroxyapatite

Nelson Torres [a], Sunho Oh [a], Mark Appleford [a], David D. Dean [b], James H. Jorgensen [c], Joo L. Ong [a], C. Mauli Agrawal [a], Gopinath Mani [a,*]

[a] Department of Biomedical Engineering, The University of Texas at San Antonio, One UTSA Circle, San Antonio, TX 78249, USA
[b] Department of Orthopedics, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229, USA
[c] Department of Pathology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229, USA

ARTICLE INFO

Article history:
Received 2 December 2009
Received in revised form 15 February 2010
Accepted 19 February 2010
Available online 25 February 2010

Keywords:
Hydroxyapatite
Self-assembled monolayers
Surface characterization
Surface modification

ABSTRACT

Open fractures are common in battlefields, motor vehicle accidents, gunshot wounds, sports injuries, and high-energy falls. Such fractures are treated using hydroxyapatite (HA)-based bone graft substitutes. However, open fracture wounds are highly susceptible to bacterial infections. Hence, this study was focused on incorporating antibacterial properties to HA using silver (Ag) carrying self-assembled monolayers (SAMs). Also, the stability of Ag carrying SAMs on HA was investigated under sterilization and physiological conditions. Initially, the –COOH terminated phosphonic acid SAMs of two different chain lengths (11 carbon atoms – shorter chain and 16 carbon atoms – longer chain) were deposited on HA. Antibacterial SAMs (ASAMs) were prepared by chemically attaching Ag to shorter and longer chain SAMs coated HA. X-ray photoelectron spectroscopy, atomic force microscopy, and contact angle goniometry collectively confirmed the attachment of Ag onto SAMs coated HA. The bacterial adhesion study showed that the adherence of *Staphylococcus aureus* was significantly reduced on ASAMs coated HA when compared to control-HA. The stability studies showed that gas plasma, dry heat and autoclave degraded most of the ASAMs on HA. UV irradiation did not damage the shorter chain ASAMs as vigorously as other treatments, while it degraded the longer chain ASAMs completely. Ethylene oxide treatment did not degrade the longer chain ASAMs unlike all other treatments but it severely damaged the shorter chain ASAMs. Both shorter and longer chain ASAMs significantly desorbed from the HA surfaces under physiological conditions although longer chain ASAMs exhibited better stability than shorter chain ASAMs. This study demonstrated the potential for using ASAMs to provide antibacterial properties to HA and the need for developing techniques to improve stability of SAMs under sterilization and physiological conditions.

1. Introduction

Bacterial infection remains an important risk factor in orthopedic surgery [1,2]. The percentage of infections estimated for knee, hip, shoulder, and elbow implants are 0.5–12%, 1–2%, 1–2.5%, and 7–9%, respectively [3]. Since millions of people receive orthopedic implants every year, even a low percentage of infection reported can cause serious complications in tens of thousands of patients. The infections are mainly caused by two bacteria, *Staphylococcus aureus* and *Staphylococcus epidermidis* [4]. After infection, these bacteria form biofilms on the implant surface and become highly resistant to systemic antibiotic treatment [4]. These infections cause debilitating pain and prolonged disability in patients. Not only it is a challenge for clinicians to re-operate at the infected site for replacing the implant but it is also an expensive treatment.

The clinical treatments currently available for orthopedic implant-associated infections are systemic therapy [5], antibiotic loaded cements [3] and antimicrobial coatings [6–8]. The toxicity of systemic agents and the difficulties in achieving right concentrations at the infected site are the drawbacks of systemic therapy [5]. In case of antibiotic loaded cements, the antibiotics are slowly released over a prolonged period, which poses a threat of causing antibiotic resistance in human body [3]. Ammonium compounds [6], iodine [7], and silver ions [8] have been previously explored as antimicrobial coatings. Silver exhibits antibacterial properties by interacting with proteins and enzymes of bacteria and causes structural damages to cell wall and bacterial membrane [9,10]. Specifically, silver binds to bacterial DNA and RNA and thereby prevents bacterial reproduction [9,10]. Also, silver binds to sulfhydryl groups of metabolic enzymes and thereby inhibits electron transport chain of the cell, which result in bacterial destruction [9,10]. Recently, self-assembled monolayers (SAMs) have been used as tethers for the attachment of silver to metals such as titanium and stainless steel [11]. Interestingly, this study showed that the silver attached to SAMs is more effective in preventing bacterial adhesion than the silver precursor itself. Hence, the

* Corresponding author. Tel.: +1 210 458 5081; fax: +1 210 458 7007.
E-mail address: Gopinath.Mani@utsa.edu (G. Mani).

1742-7061/$ - see front matter © 2010 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2010.02.032

means by which the silver is presented may play a critical role in imparting effective antibacterial properties to a material.

Bacterial infections are especially more prevalent in open fractures that result from trauma [12]. Such injuries often involve the loss of bone and the resulting defects have to be augmented with grafts or synthetic bone graft substitutes. Hence, there is a great need for the development of a coating system that can provide antibacterial properties to commonly used synthetic bone graft substitutes. The currently available synthetic bone graft substitutes can generally be classified under two different categories: (a) ceramic-based bone grafts; (b) polymer-based bone grafts [13]. Ceramic-based bone grafts include the use of calcium phosphate, calcium sulfate, and bioglass. Calcium phosphates are commonly used for this application and it mainly includes synthetic hydroxyapatite and tricalcium phosphate [13]. Since the primary inorganic component of bone is calcium hydroxyapatite, the use of calcium phosphates has a slight edge over the use of other materials for making bone grafts [13]. Approximately, 60% of the currently available bone grafts involve the use of ceramics either by itself or in combination with another material [13]. A wide variety of polymers, including both degradable and biodegradable, has also been extensively used for making bone grafts [13].

Hydroxyapatite (HA), a synthetic bone substitute material [14,15], is also used to coat metal implants to improve their osseointegration [16,17]. However, HA is also prone to bacterial infection like any other material [18,19]. In this study, the use of a silver carrying self-assembled monolayer coating was explored for imparting antibacterial properties to hydroxyapatite. The stability of silver-attached SAMs on HA was investigated under five commonly used sterilization methods and also under physiological conditions for potential biomedical applications.

2. Materials and methods

Acetone and absolute ethanol (200 proof) were purchased from Pharmco-Aaper (USA). Methanol, 11-phosphonoundecanoic acid, 16-phosphonohexadecanoic acid, stearic acid, and silver nitrate were all purchased from Sigma–Aldrich (USA). Anhydrous tetrahydrofuran (THF) was purchased from Alfa Aesar. All reagents were used as received. Dense circular hydroxyapatite tablets (9.5 mm diameter × 2.4 mm thick) were purchased from HiMed (Old Bethpage, NY). The specific density, bulk density, crystallinity, sintering temperature, and surface roughness of the hydroxyapatite tablets were obtained from HiMed and are included in Table 1. The grain sizes of control-HA were determined from the scanning electron microscopy (SEM) and atomic force microscopy (AFM) images (Fig. 1) and included in Table 1 as well.

2.1. Preparation of HA specimens

As-received HA specimens were chemically cleaned by sonication in ethanol, acetone, and methanol for 10 min (twice) each.

Table 1
Characterization of HA tablets used in this study.

Parameters	Values
Phase composition	>95% hydroxyapatite (by X-ray diffraction)
Specific density	3.1 ± 0.1 g/cc (by helium pycnometer)
Bulk density	2.8 ± 0.2 g/cc (by weight/volume)
Crystallinity	100% (by X-ray diffraction)
Grain size	2–6 μm (by SEM and AFM)
Sintering temperature	1150–1200 °C
Surface roughness (Ra)	1.0 ± 0.3 μm (by Taylor–Hobson Surtronic 3P profilometer)

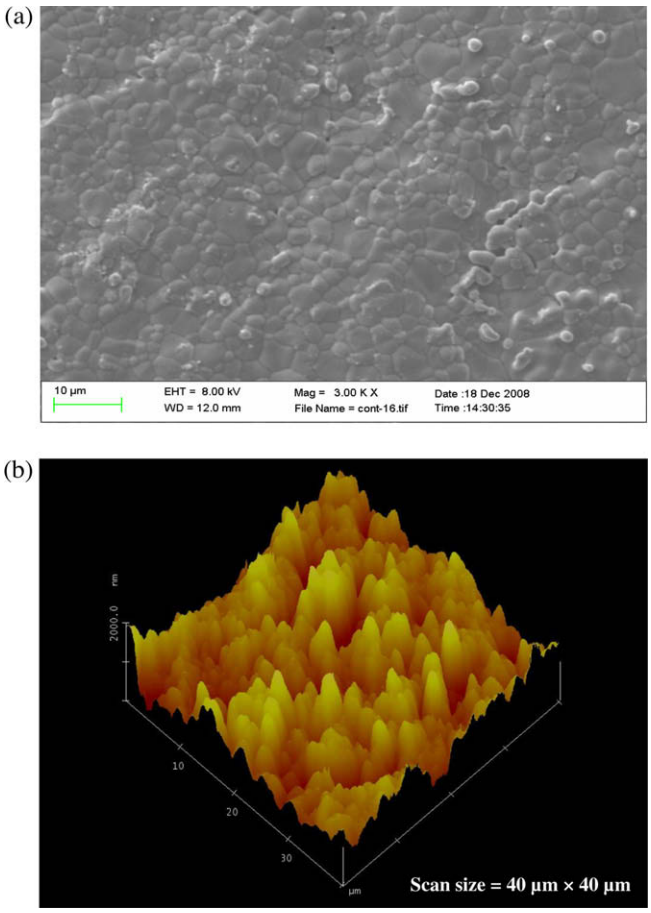

Fig. 1. SEM image (a) and AFM tapping mode height 3D image (b) of control-HA.

The specimens were then dried under a stream of nitrogen (N_2) gas. Two sets of control-HA specimens were prepared. The first set consisted of chemically cleaned HA specimens and are referred to here as control–HA–1. In the second set, the HA specimens were subjected to the SAM deposition protocols described below but without the addition of SAM-forming molecules and are referred to here as control–HA–2.

2.2. Formation of –CH_3 terminated SAMs on HA surfaces

The rationale for using –CH_3 terminated molecules in this study is to optimize the formation of SAMs on HA using a simple static contact angle measurement technique. The contact angles of –CH_3 terminated monolayer coated substrates are hydrophobic and show much higher contact angle than that of uncoated (control) substrates which are typically hydrophilic [20–23]. This significant change in the wettability property has been attributed to the successful formation of SAM on variety of materials including metals and ceramics [20–23].

Octadecylphosphonic acid (OPA) and stearic acid (SA) were the two –CH_3 terminated molecules used in this study to deposit SAMs on HA. Two different types of SAM deposition methods were carried out at room temperature (RT) and boiling temperature (BT). For RT deposition, the chemically cleaned HA specimens were immersed in 3 ml of 1 mM solution of OPA or SA in anhydrous THF at room temperature for 18 h followed by annealing the specimens at 120 °C for 18 h without rinsing. The specimens were then cleaned using THF and double-distilled water (dd-H_2O) for 1 min each followed by N_2 gas drying. For BT deposition, the HA specimens were initially cooled in an ice bath for 1 h followed by heating