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ScienceDirect
Acta Biomaterialia 5 (2009) 1425–1432
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Reduced responses of macrophages on nanometer surface features of altered alumina crystalline phases

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Received 3 July 2008; received in revised form 31 October 2008; accepted 26 January 2009
Available online 1 February 2009

Abstract

Extensive prolonged interactions of inflammatory cells (such as macrophages) at the host–implant interface may lead to implant failure. While previous studies have shown increased in vitro and in vivo bone cell adhesion, proliferation and mineralization on nanophase compared to currently implanted ceramics, few studies have been conducted to elucidate inflammatory cell responses on such nanophase ceramics. Controlling surface feature size and corresponding surface roughness on implants may clearly alter immune cell responses, which would be an extremely important consideration for the use of nanostructured materials as improved biomaterials. In this study, reduced macrophage density was observed on alumina (Al_2O_3) compacts with greater nanometer surface roughness accompanied by changes in crystallinity for up to 24 h in culture. Since alumina is a commonly used ceramic in orthopedic applications, this in vitro study continues to support the use of nanophase ceramics as improved orthopedic implants by demonstrating reduced macrophage responses.

Keywords: Bone; Alumina; Macrophage; LPS; Inflammation

1. Introduction

Alumina (Al_2O_3) has been used in orthopedic and dental implants because, like other ceramics, it possesses desirable mechanical properties such as compressive strength. In addition, the oxide surface allows alumina to be chemically inert and resistant to physical and chemical changes inside the body [1–3]. However, orthopedic implants composed of traditional ceramics, or those with micron grain sizes, sometimes fail during long term applications [4]. This is often due to poor initial bonding of implants to juxtaposed bone. Insufficient bonding between an implant and surrounding bone can be caused by extensive formation of soft fibrous tissue at the bone–implant interface, a lack of initial osseointegration or osteolysis due to wear debris between the implant and the surrounding bone [5].

Many approaches have been taken to extend the longevity of implant devices in the body so that patients who receive them would not require revision surgeries later in their lifetime [4]. Among them, nanotechnology has been applied to improve the cytocompatibility properties of orthopedic implant materials. Nanotechnology has been defined as using materials composed of grain sizes of 1–100 nm in at least one dimension. It has been shown that nanophase alumina enhances in vitro osteoblast (bone forming cells) adhesion (52% after 4 h), proliferation (14% from 1 to 5 days) and subsequent functions (i.e. four times more calcium deposition after 28 days) compared with conventional alumina [6,7]; these results have recently been confirmed in vivo by the demonstration of greater rat calvarial bone infiltration into tantalum scaffolds coated with nanophase compared to conventional ceramics after 2

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1742-7061/$ - see front matter
doi:10.1016/j.actbio.2009.01.031

weeks [6]. It is believed that nanophase materials exhibit such proactive bone growing properties since they mimic the natural nanometer grain size of bone and possess unique surface energetics to increase the adsorption of proteins (such as vitronectin) which mediate osteoblast adhesion.

In this manner, it is well known that many proteins, including fibronectin and vitronectin, mediate cell adhesion, migration, proliferation and long-term functions, and that such proteins absorb in higher quantities on higher surface energy nanophase compared to conventional ceramics [8–10]. Therefore, it is not surprising that osteoblasts adhere and proliferate more on ceramics (such as alumina, titania and hydroxyapatite) with grain sizes smaller than (compared to larger than) 100 nm [11]. Interestingly, due to the same altered initial protein adsorption events, it has been reported that fibroblast adhesion was reduced up to three times on nanophase compared to conventional alumina [11].

However, it is important to keep in mind that orthopedic surgery causes trauma to the local tissue triggering a wound healing response activating numerous inflammatory cells at the implant site long before osteoblasts arrive [12]. In addition, inflammatory cell activation at the orthopedic implant site may occur by the presence of bacteria. One type of inflammatory cell (macrophages) migrates to the bone–implant interface and becomes activated soon following implantation [12]. Macrophages clearly form the first line of defense against bacteria, viruses and foreign implanted materials [13]. While it is under debate whether researchers should design implant surfaces to promote or inhibit macrophage responses, it is clear that excessive macrophage functions leading to large amounts of persistent inflammatory tissue may be detrimental to implant performance [14,15]. For example, the overactivation of macrophages can lead to the production of proinflammatory cytokines (IL-1β, IL-6), chemokines (CCL22), matrix enzymes (elastase) and other substances (PGE2), which not only can cause osteolysis but can also stimulate the proliferation of fibroblasts [5,16] – all events which would decrease the ability of osteoblasts to attach, proliferate and form bone on orthopedic implants. Therefore, it may be key to design orthopedic implants to down-regulate macrophage activities so that wound healing can occur quickly and bone growth and mineral deposition by osteoblasts are promoted shortly thereafter [17].

However, despite this importance, no studies to date have been conducted evaluating the macrophage response on nanophase compared to conventional ceramics. For this reason, functions of unactivated and activated macrophages (including adhesion, proliferation and morphology) on nanophase compared to conventional alumina were investigated in this in vitro study. Lipopolysaccharide (LPS), a major component of the outer membrane of Gram-negative bacteria which may infect newly implant orthopedic materials, has been shown to stimulate macrophage functions in vitro [18,19]. Therefore, LPS was used in the present study as one way to create a pro-inflammatory environment which mimics in vivo environments following orthopedic implantation (such as infection) [20]. The current study provided the first evidence of reduced macrophage density on nanophase compared to conventional alumina due to altered nanoscale surface roughness and altered crystalline phases [21].

2. Materials and methods

2.1. Substrate preparation

Alumina (Al_2O_3) disks were prepared by compacting nanophase (45 nm grain size) alumina (70:30 = δ:γ-phase) powders (Alfa Aesar) in a tool-steel die via a uniaxial pressing cycle (0.2–1 GPa over a 10 min period). The final disks had 12 mm diameters and were 2 mm thick. Nanophase alumina samples were obtained by sintering, in air at 10 °C min^{-1}, the 45 nm grain size alumina compacts from room temperature to either 1000 or 1100 °C and by maintaining that temperature for 2 h. Microphase (also called conventional grain size) alumina samples were obtained by sintering (in air at 10 °C min^{-1}) the 45 nm grain size alumina compacts from room temperature to 1200 °C and by maintaining this temperature for 2 h [22]. All samples were sterilized with ultraviolet light for 15 min immediately before cell experiments.

2.2. Surface characterization

Sintered conventional and nanophase alumina samples were mounted on stainless steel stubs and sputter-coated with gold (10 nm thickness) prior to examination using a Leo 1530-VP scanning electron microscope (SEM). Grain size was determined by the linear intercept method [23]. Nanometer surface topography was measured using a multimode atomic force microscope (AFM; Nanoscope IIIa, Veeco, CA) with scan areas of 3 μm×3 μm. Commercially available AFM tips (radius of tip curvature was less than 10 nm, NSC15/ALBS, Micro-Masch, OR) were used in tapping mode (tip height was 25 μm with a 40 N m^{-1} force constant) with a scanning rate of 10 Hz. Surface topography was evaluated on randomly selected areas in 3 μm × 3 μm AFM scans at least three times (on one substrate for each group) using computer software (Nanoscope 4.42, Veeco, CA).

2.3. Surface crystallinity

X-ray diffraction (XRD) graphs were obtained using a Siemens Diffracktometer D5000 with Cu K_α radiation at 40 kV and 35 mA. Scanning was recorded on a KEVEX detector between 0° (2θ) and 90° (2θ) with a 1.2° min^{-1} scan speed, and standard JCPDS XRD patterns from Diffrac Plus XRD Commander Software (Bruker Advanced X-Ray Solutions) were used to confirm the phases present in the alumina samples. The scanning was repeated for three samples in each group. The results were analyzed with a Diffracplus Basic 4.0 XRD evaluation program.

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