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Abnormal arrangement of a collagen/apatite extracellular matrix orthogonal to osteoblast alignment is constructed by a nanoscale periodic surface structure

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ABSTRACT

Morphological and directional alteration of cells is essential for structurally appropriate construction of tissues and organs. In particular, osteoblast alignment is crucial for the realization of anisotropic bone tissue microstructure. In this article, the orientation of a collagen/apatite extracellular matrix (ECM) was established by controlling osteoblast alignment using a surface geometry with nanometer-sized periodicity induced by laser ablation. Laser irradiation induced self-organized periodic structures (laser-induced periodic surface structures; LIPSS) with a spatial period equal to the wavelength of the incident laser on the surface of biomedical alloys of Ti–6Al–4V and Co–Cr–Mo. Osteoblast orientation was successfully induced parallel to the grating structure. Notably, both the fibrous orientation of the secreted collagen matrix and the *c*-axis of the produced apatite crystals were orientated orthogonal to the cell direction. To the best of our knowledge, this is the first report demonstrating that bone tissue anisotropy is controllable, including the characteristic organization of a collagen/apatite composite orthogonal to the osteoblast orientation, by controlling the cell alignment using periodic surface geometry.

1. Introduction

The realization of *in vitro* tissue construction shows great potential not only for tissue regeneration but also for understanding the mechanisms that regulate tissue genesis. Living tissues and organs express specialized forms and unique physical properties required to carry out their specific functions. Bone tissue, in particular, has a characteristic hierarchical anisotropic structure, from nano- to macroscale [1], which governs the mechanical properties of bone tissue [2]. Regeneration of bone that maintains the original anisotropic structure requires specially-designed biomaterials that can retrieve the crystallographic orientation of bone, because even advanced tissue engineering techniques cannot promote anisotropic regeneration of bone tissue [3,4]. Control of the orientation of cell-produced collagen/apatite in the favored direction is therefore essential in bone tissue engineering.

Patterning of extracellular matrix (ECM) deposition is considered to be closely related to polarized cell behaviors during morphogenesis [5], and the interplay between integrin receptors and the ECM is thought to be important for a variety of morphogenetic events [6]. Directional deposition of matrix proteins corresponding to cell orientation has been demonstrated using microgrooves [7–9] and an anisotropic mechanical environment [10], indicating that the anisotropy of the ECM is determined by cellular arrangement in response to external stimuli. Indeed, that “the cell-produced matrix orientation follows the cellular direction” is a classical belief of tissue matrix structure formation. However, it is not known this always holds true.

In the present article, osteoblasts were grown on a biomimetic nanogrooved substrate, and the architecture of the resulting bone matrix was examined both qualitatively and quantitatively. Laser-induced nanometer-scale surface topography was introduced into two typical biomedical alloys, Ti–6Al–4V and Co–Cr–Mo. The cells

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preferentially aligned along the nanogrooves, consistent with the results of previous studies [11,12]. In this study, however, the architecture of the cell-produced bone matrix, which was quantitatively determined using Raman microscopy and microbeam X-ray diffraction (XRD), showed unique anisotropy against the cellular orientation; these findings reverse the current belief that the ECM is constructed parallel to the cellular arrangement and provide new insight into the mechanisms of bone tissue morphogenesis. These findings on the relationship between osteoblasts and bone matrix orientation will facilitate innovation in the development of biomaterials appropriate to induce bone regeneration, including recovery of the original anisotropic structure of bone, and may also facilitate breakthroughs in our understanding of the mechanism underlying anisotropic tissue generation.

2. Materials and methods

2.1. Fabrication of periodic surface structures

Samples of forged Ti–6 mass% Al–4 mass% V alloy (Ti–6Al–4V; ASTM F136-02A) and Co–28 mass% Cr–6 mass% Mo alloy (Co–Cr–Mo; ASTM F1537-08) with a diameter of 15 mm and a height of 5.0 mm were obtained. These samples were ground using emery paper (#120, #320, #600), then polished with diamond paste (9 and 3 μm) and colloidal silica suspension (0.06 μm). Periodic structures were generated on the surface of each sample using a p-polarized Ti: sapphire femtosecond laser (peak wavelength of 800 nm, pulse width of 250 fs, cyclic frequency of 2 kHz). A circularly polarized laser was used to prepare a control substrate without directional characteristics.

2.2. Surface characterization

The periodic surface structures produced by laser irradiation were observed using scanning electron microscopy (SEM; VE-7800, Keyence, Osaka, Japan). The

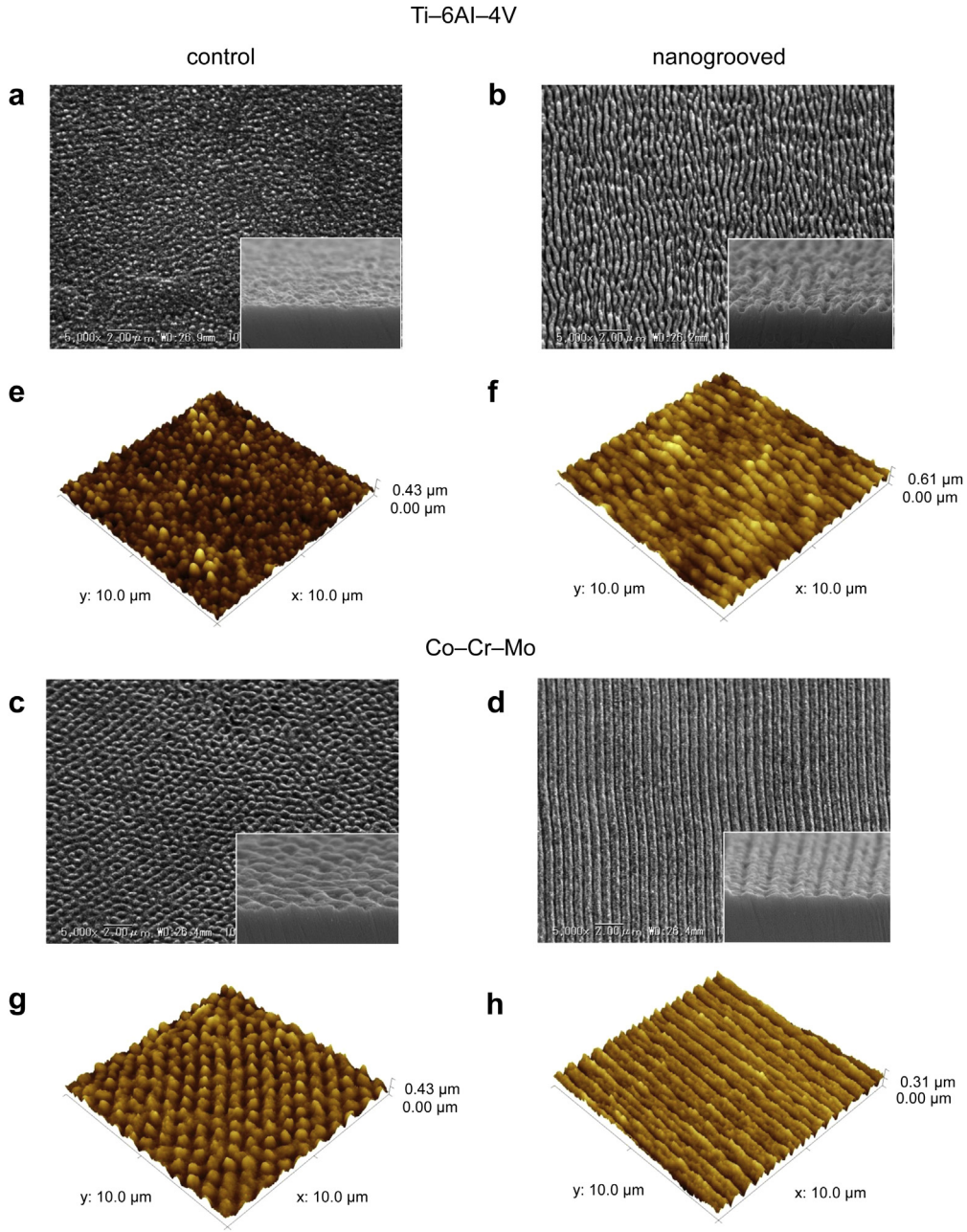

Fig. 1. SEM images ((a)–(d)) and AFM images ((e)–(h)) of the surface topography of Ti–6Al–4V ((a), (b), (e), (f)) and Co–Cr–Mo ((c), (d), (g), (h)). The inset outlines the cross-sectional SEM image of each alloy. The control surfaces display isotropic geometry with a fine-dot structure ((a), (c), (e), (g)), whereas the laser-irradiated substrates display anisotropic periodicity with spatial periods somewhat smaller than the wavelength of the incident laser, at 800 nm ((b), (d), (f), (h)).