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Osteointegration of titanium implant is sensitive to specific nanostructure morphology

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ABSTRACT

An important aspect of orthopedic implant integration is the enhancement of functional activity of osteoblasts at the tissue–implant interface without any fibrous tissue intervention. Nanostructured implant surfaces are known to enhance osteoblast activity. Previously, we have reported a simple hydrothermal method for the fabrication of non-periodic nanostructures (nanoscaffold, nanoleaves and nanoneedles) on titanium implants showing good biocompatibility and a distinct osteoblast response in vitro in terms of osteoblast adhesion to the surface. In the present work, these nanostructures have been evaluated for their detailed in vitro cellular response as well as in vivo osteointegration. Our studies showed that a specific surface nanomorphology, viz. nanoleaves, which is a network of vertically aligned, non-periodic, leaf-like structures with thickness in the nanoscale, provided a distinct increase in osteoblast cell proliferation, alkaline phosphatase (ALP) activity and collagen synthesis compared to several other types of nanomorphology, such as nanotubes, nanoscaffold and nanoneedles (rods). Gene expression analysis of ALP, osteocalcin, collagen, decorin and Runx2 showed ~20- to 40-fold up-regulation on the leaf-like topography. Cytoskeletal arrangement studies on this substrate again revealed a unique response with favorable intracellular protein expressions of vinculin, FAK and src. In vivo osteointegration study over 12 weeks on rat model (Sprague–Dawley) showed early-stage bone formation (60% bone contact by week 2 and ~85% by week 8, $p < 0.01$) in the leaf-like nanopattern, without any inflammatory cytokine production.

1. Introduction

The success of any orthopedic or dental implantation procedure is based on the formation of an effective interface between the surface of the implant material and the bone tissue, without any fibrous tissue intervention [1]. Current orthopedic implants are limited by the lack of appropriate cell adhesion and osteointegration, leading to reduced implant lifespan. Improvements in implant surface topography as well as surface chemistry have been established as ways to improve bone bonding [2–4]. Recent efforts in this field have highlighted the importance of nanotechnology in altering the surface topography of materials such as metals, ceramics, polymers and composites to better mimic the surface roughness features of natural bone [5–10]. The principle behind surface structural modifications on implants at the nanoscale is that such surfaces would mimic the extracellular matrix with which cells normally interact and hence would favor positive interaction with cells [11]. Indeed, this principle has been verified in several cases. Various studies have shown that surface energy and nanotopography influence the type, quantity and conformation of adsorbed protein, and control cellular adhesion to the surface [12–17]. Specifically, the active site of vitronectin (RGD sequence) has been found to be more exposed on nanophase ceramics than on conventional ceramics [7,18]. Studies on lithographically patterned nanofeatures have shown that a periodic pattern of 400 nm dots enhanced osteoblast differentiation of human mesenchymal stem cells (hMSCs), but not when cultured on 150 and 600 nm dot patterns [19]. Similarly, periodic, vertically arranged nanotube arrays, with less than 30 nm spacing and varying pore diameters (<100 nm), enhanced osteoblast cell functions [20], as well as endothelial cell proliferation without vascular smooth muscle proliferation [21]. Dalby et al. [5], in a detailed investigation on patterned polymeric surfaces with disordered arrangement of dots in square arrays, having a displacement of 50 nm between dots, showed enhanced osteoblast differentiation of hMSCs compared to an ordered substrate. All these reports highlight the significance of nanoscale substrate topographies in controlling cellular response.

In our previous study [22], we reported a simple, scalable, inexpensive and one-step wet chemical (hydrothermal) method for the

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