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Morphometric immunohistochemical examination of the inflammatory tissue reaction after implantation of calcium phosphate-coated titanium plates in rats

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Abstract

Calcium phosphate (CaP) preparations are established coatings for titanium-based medical implants used for bone reconstruction. However, biodegradation of the coating can result in microparticles that subsequently cause inflammatory reactions. The present study was therefore aimed at investigating the inflammatory response to two series of CaP-coated titanium plates: Ti-brushite (Ti-B) and Ti-hydroxyapatite (Ti-H) implants. Fifteen male LEW.1A rats received one plate of each series and a pellet (5 × 2 mm) of sol–gel derived silica/CaP (SCP implants) implanted into the back musculature. After 7, 14 and 28 days, five rats were killed and the implants were removed with the surrounding tissue. Quantitative immunohistochemistry was performed on frozen sections. Total monocytes/macrophages, tissue macrophages, T-cells, MHC-class-II-positive cells and proliferating cells were counted. For the Ti-B implants, the number of monocytes/macrophages remained constant while the other cell populations increased. In contrast, for the Ti-H implants the number of monocytes/macrophages decreased while the other cell populations remained constant. The SCP implants demonstrated degradation and scattering into smaller particles with an increase for all cell populations except the proliferating cells. Human mesenchymal stem cells demonstrated adherence and a flat morphology on Ti-B and Ti-H implants and no remarkable difference between both implants. Taken together, the in vivo data demonstrate that the short-term inflammatory response against a hydroxyapatite coating is lower in comparison to a brushite coating, and that the morphology of cells growing in vitro is similar on both layers.

Keywords: Implantation; Hydroxyapatite; Calcium phosphate; Inflammation; Cell adherence

1. Introduction

The regeneration of bone that has been destroyed by injury or disease is a challenge in orthopaedic, reconstructive and dental surgery. Implantation of autologous grafts is the preferred method for reconstruction and repair of bone lesions [1]. However, for stabilization of such defects the use of artificial implants, at present preferably made of titanium, is necessary. Calcium phosphate (CaP)-based preparations are among the most commonly used materials for filling bone defects and for ceramic coating of metallic implants used as support devices in bone fracture treatment and to replace diseased bone segments [2,3].

CaP as a natural substance is the main component of the mineral matrix of bone and typically constitutes 60% of the bone weight. Chemically, it exists in a variety of different

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preparations which differ in the atomic and ionic lattice configuration, Ca:P ratio, the number and size of pores, and surface area. These factors influence the stability of the material and subsequently its behaviour and effects in the biological host environment. Apart from natural CaP preparations derived from coralline material, hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$), brushite (dicalcium phosphate dihydrate (DCPD), $CaHPO_4{\cdot}2H_2O$) and tricalcium phosphate (TCP, $Ca_3(PO_4)_2$) are among the most commonly used forms of CaP. As a biomaterial, CaP is generally considered to be biocompatible, as well as bioactive due to its beneficial interactions with bone [4]. It possesses osteoinductive properties and can therefore be used as a scaffold to induce the growth of osseous tissue. This is an important point for integration and long-term fixation of implants in bone [5]. Clinical use in human surgery began in the 1980s with hip replacement implants coated with HA.

Recent long-term follow-up studies, as well as case reports on CaP-coated medical implants after explantation due to reasons other than implant failure, indicate that CaP coatings exhibit good long-term functionality [6–9]. However, the rate of resorption has to be matched with the rate of bone tissue growth to achieve optimal stability. An excess of resorption might lead to a chronic surplus supply of calcium and phosphate ions in the body, and biodegradation of CaP implants and coatings might result in microparticles. Several case studies report dissolution and the occurrence of scattered CaP particles in the tissue around implants which were explanted after several years [10,11]. There are indications that the deposition of CaP microparticles is involved, for example, in arthritis, kidney inflammation and connective tissue diseases [12–15]. Chronic inflammation was also found in some unusual cases of pyogenic granuloma following HA implant exposure [16]. Furthermore, CaP is used as an adjuvant in vaccines, indicating its potency to induce or enhance immunological reactions [17].

As with all biomaterials, the inflammatory response of the host has to be considered as a prime factor in the context of the biocompatibility of CaP coatings, influencing their short- and long-term stability and therefore the clinical success of respective implants. Using in vitro studies, CaP microparticles have been shown to induce pro-inflammatory cytokines, thereby mediating possible systemic effects and long-term chronic inflammation [18,19]. However, cell culture experiments might provide some information but are limited regarding their relevance because inflammation involves complex interactions between different cell types. On the other hand, there are few in vivo studies investigating the inflammatory response following implantation of CaP or CaP-coated implants. Almost all of these studies provide only a qualitative judgement or a semi-quantitative scale for the assessment of the inflammatory reactions, and very few studies provide a detailed differentiation of the cell populations involved. Furthermore, there are conflicting results [5,20–24]. It has also been demonstrated that the inflammation reaction to CaP implants depends on the internal porosity as well as the shape and size of the material [25–28]. Additionally, it can be assumed that the choice of animal model and implantation site also influences the extent and the time course of the inflammatory response. One recent study used a quantitative approach to examine the short-term response investigating the number of giant cells, macrophages and total lymphocytes [29].

The present study was aimed at a quantitative evaluation of five different cell populations which are involved in the short-term inflammatory reaction following intramuscular implantation of titanium plates coated with two different CaP coatings: brushite and HA. As the rate of dissolution of CaP decreases with increasing Ca:P ratio, the hypothesis behind this approach was that a HA coating reduces the inflammatory response because the Ca:P ratio of HA is 1.67 in comparison to that of brushite of 1. Furthermore, a clinically approved CaP granulate consisting of BONITmatrix® with a Ca:P ratio of 1.6 [30] has been used to evaluate the influence of the form of the CaP preparation, coating vs. granulate, on the inflammatory response.

In addition to the histological examination of the inflammatory reaction, a visual examination of the morphology of human mesenchymal stem cells derived from bone marrow after in vitro growth on the surfaces of the CaP-coated implants was performed. The aim of these experiments was to assess the suitability of the surface for adequate cell adhesion and spreading and to determine the influence of different CaP phases on these parameters.

2. Materials and methods

2.1. Animal experiments

2.1.1. Laboratory animals

Fifteen male Lewis rats (LEW.1A; 100 days old, median weight 370 ± 44 g) were used in the experiments. They were bred and maintained in our in-house facilities under conventional housing and feeding conditions. All aspects of the animal experiments were conducted in accordance with the animal protection law of the Federal Republic of Germany in its new version of 1 January 1987, with the principles of care for animals in laboratories (drawn up by the National Society for Medical Research) and with the Guidelines for Keeping and Using Laboratory Animals (NIH Publication No. 80-23, revised 1985).

2.1.2. Implants

Titanium plates $5 \times 5 \times 1$ mm with either a layer of brushite (designated Ti-B implants) or a layer of HA (designated Ti-H implants) were used. In a first step, all implants were coated with BONIT® (brushite, Ca:P ratio 1.0). The coating was performed at room temperature via a proprietary electrochemical deposition process (patent WO 0205862) which results in a layer thickness of about 20 μm with a fine crystalline surface structure and a porosity of about 60%. The physical and chemical characteristics of the BONIT® coating have been described in detail previously [30]. In a second step for the Ti-H implants only, this

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