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Osteoconductive modifications of Ti-implants in a goat defect model: characterization of bone growth with SR μCT and histology

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Abstract

In this work the osteoconductive potential of coatings for titanium implants using different extracellular matrix components was evaluated. Cylindrical implants with two defined cavities A and B were coated with collagen type I, type III, or RGD peptide, and placed in the femur of goats together with an uncoated reference state. Bone contact and volume were determined after 5 and 12 weeks implantation, using both histomorphometry and synchrotron radiation micro computed tomography (SR μCT) as the methods complement each other: SR μCT allows for a high precision of bone detection due to the large number of analysed slices per sample, while histology offers a better lateral resolution and the possibility of additionally determining bone contact.

Both methods revealed similar tendencies in bone formation for the differently bio-functionalized implants, with the SR μCT data resulting in significant differences. After 5 and 12 weeks, all three coatings showed a significant increase in bone volume over the uncoated reference, with the highest results for the collagen coatings. The coating consisting of just the RGD-sequence to improve cell adhesion showed only a slight improvement compared with the reference material.

For uncoated titanium, RGD, and especially collagen type I, the response in cavity A, situated in denser bone, was stronger than in cavity B. Collagen type III, on the other hand, appeared to be the more effective coating in areas of lesser bone density as represented by cavity B. These results indicate that matrix molecules (or combinations thereof) are capable of generating the appropriate signals for the specific microenvironment around implants and can thus accelerate the bone formation process and increase the stability of implants.

Keywords: Titanium; Collagen type I; Collagen type III; RGD peptide; Osseointegration; Histomorphometry; Synchrotron radiation; Micro computed tomography

1. Introduction

The integration of titanium implants into host bone is already comparatively good, based on periimplant bone formation and the occurrence of direct bone anchorage. However, due to the ageing of our population, there is an increasing group of patients with challenging general health and bone conditions where implants are more prone to failure. Therefore, there is need for simple methods that improve the periimplant bone formation and the short- and long-term implant stability in applications like dental implants and orthopaedic prostheses.

As the interaction of cells with the implant surface determines periimplant bone regeneration to a large part, the preparation of biomaterials with advantageous

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biochemical surface properties [1] is receiving increasing attention.

One strategy is the immobilization of extracellular matrix components (native structural proteins, peptide sequences [2,3], or synthetic molecules based on matrix molecules [4,5]) since the ECM provides both a scaffold for cell adhesion and takes part in regulating cellular functions [6]. Collagen type I is the predominant extracellular matrix (ECM) component in vivo, and due to its low immunogenicity and high conformational stability eminently suitable for the surface engineering of implants. Experiments using primary rat calvaria osteoblasts have shown that such coatings favor the adhesion and differentiation of osteoblastic cells [7]. As the ECM is highly diverse for different tissues or stages of development, other components can also be of interest due to their function in specific situations. One such situation is fracture healing, where initially collagen type III is expressed, which forms a scaffold for the migration of osteoprogenitor cells as well as capillary ingrowth and is only later replaced by collagen type I [8,9].

A second method in the surface modification of implants makes use of peptide sequences. Such sequences can consist of parts of adhesion molecules possessing specific functions: One example is the RGD sequence, which specifically mediates cellular adhesion via integrin receptors like $\alpha 2\beta 1$, $\alpha IIb\beta 3$, and $\alpha v\beta 3$.

For both native molecules and peptides, the immobilization process is an important aspect in creating surfaces with defined properties. Covalent fixation has been used almost exclusively for metallic biomaterials [10], but large and conformationally stable proteins like fibrillar collagen can be immobilized by a purely physisorptive process, with the resultant protein layer being stable against competitive adsorption of serum proteins [11]. For small peptides physisorption is not the optimal process due to their higher tendency to desorb, but using valve metals [12] such as titanium the adsorbed peptides can be partially incorporated into anodically formed oxide layers through electrochemical methods [13,14]. Compared to classical covalent coupling, the main advantages of both methods are a single step procedure and process conditions with physiological parameters, allowing for the immobilization of conformationally labile biomolecules.

The bone formation process around implants is usually characterised using light microscopy, which gives an excellent lateral picture resolution and quantitative data through histomorphometry. The information from various sections is extrapolated to the represent the entire sample. However, such an extrapolation can result in incorrect information, since loss of large tissue quantities (up to 300 μm) is not uncommon during the sectioning of hard tissue-implant samples.

Micro computed tomography (μCT) may be an alternative, as it has already been shown to be a very powerful technique for the quantification of mineralized bone [15,16]. By means of this method the bone formation can be determined with high spatial resolution in a non-destructive way. Using synchrotron radiation micro tomography (SR μCT) [17] with its monoenergetic and nearly parallel X-rays, problems (such as scattering) arising from the highly divergent attenuation coefficients of bone and titanium can be avoided [18]. This leads to a higher sensitivity of bone detection near the implant surface.

It is the aim of this work to quantify the bone response around implant surfaces that create a cellular microenvironment beneficial to implant integration. To this end ECM components either specific to the target tissue (collagen type I, type III) or to certain functions like cell adhesion (RGD-peptide) have been used. In this study we investigated the osteoconductive potential of the coatings (i) fibrillar collagen type I and (ii) fibrillar collagen type III, both immobilized by physisorption, as well as (iii) cyclic RGD-peptide with phosphonic acid anchor groups [2,19] immobilized electrochemically assisted by partial incorporation into the anodic oxide layer on titanium implants [13] in a goat defect model [19]. The chosen implant geometry [8] offered the possibility to quantify the osteoconductive potential of the surface states in defined gap regions. For analysis both SR μCT and histological methods were used.

2. Material and methods

2.1. Design and coating of implants

Cylindrical titanium implants according to Fig. 1 with a diameter of 4 mm and a length of 12 mm were used. To clearly define a region of bone formation, two incisions with a width of 2.5 mm and a depth of 0.7 mm were cut into the titanium surface.

The implants were sandblasted with 250 μm corundum and cleaned with 1% Triton X-100, acetone, and 96% ethanol, rinsed with distilled water, and air dried.

Employed components for surface coating were acid soluble bovine skin collagen type I (Fluka, Deisenhofen,

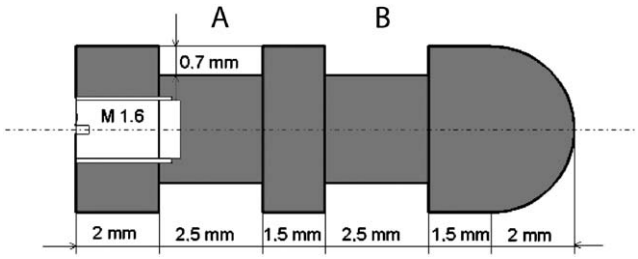

Fig. 1. Scheme of the investigated Ti-implants in a goat model. The cavities in the cylindrical implant are used as a defined volume for the analysis of bone growth differences, depending on the titanium surface modification.