Acta Biomaterialia 10 (2014) 2855–2865

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Increased bone remodelling around titanium implants coated with chondroitin sulfate in ovariectomized rats

Jan Dudeck [a,b,*], Sebastian Rehberg [a], Ricardo Bernhardt [c], Wolfgang Schneiders [a], Oliver Zierau [d], Manjubala Inderchand [e,g], Jürgen Goebbels [f], Günter Vollmer [d], Peter Fratzl [g], Dieter Scharnweber [c], Stefan Rammelt [a,h]

[a] Department of Trauma and Reconstructive Surgery, University Hospital "Carl Gustav Carus", Technische Universität Dresden, Fetscherstrasse 74, Dresden 01307, Germany
[b] Institute of Immunology, Medical Faculty "Carl Gustav Carus", Technische Universität Dresden, Fetscherstrasse 74, Dresden 01307, Germany
[c] Max Bergmann Center of Biomaterials, Technische Universität Dresden, Budapester Str. 27, Dresden 01069, Germany
[d] Institute for Zoology, Chair for Molecular Cell Physiology and Endocrinology, Technische Universität Dresden, Zellescher Weg 2b, Dresden 01062, Germany
[e] Biomedical Engineering Division, School of Bio-Sciences and Technology, VIT University, Vellore, Tamilnadu 623014, India
[f] BAM Federal Institute for Materials Research and Testing, Unter den Eichen 87, Berlin 12205, Germany
[g] Max Planck Institute of Colloids and Interfaces, Department of Biomaterials, Potsdam-Golm Science Park, Am Mühlenberg 1, Potsdam 14476, Germany
[h] Center for Regenerative Therapies Dresden (CRTD), Tatzberg 47, Dresden 01307, Germany

ARTICLE INFO

Article history:
Received 21 June 2013
Received in revised form 25 January 2014
Accepted 31 January 2014
Available online 15 February 2014

Keywords:
Osseointegration
Scanning nanoindentation
Chondroitin sulfate
Osteoporosis
Estrogen replacement therapy

ABSTRACT

Coating titanium implants with artificial extracellular matrices based on collagen and chondroitin sulfate (CS) has been shown to enhance bone remodelling and de novo bone formation in vivo. The aim of this study was to evaluate the effect of estrogen deficiency and hormone replacement therapy (HRT) on the osseointegration of CS-modified Ti implants. 30 adult female, ovariectomized Wistar rats were fed either with an ethinyl-estradiol-rich diet (E) to simulate a clinical relevant HRT or with a genistein-rich diet (G) to test an alternative therapy based on nutritionally relevant phytoestrogens. Controls (C) received an estrogen-free diet. Uncoated titanium pins (Ti) or pins coated with type-I collagen and CS (Ti/CS) were inserted 8 weeks after ovarectomy into the tibia. Specimens were retrieved 28 days after implantation. Both the amount of newly formed bone and the affinity index ($P < 0.05$) were moderately higher around Ti/CS implants as compared to uncoated Ti. The highest values were measured in the G-Ti/CS and E-Ti/CS groups, the lowest values for the E-Ti and G-Ti controls. Quantitative synchrotron radiation micro-computed tomography (SRμCT) revealed the highest increase in total bone formation around G-Ti/CS as compared to C-Ti ($P < 0.01$). The effects with respect to direct bone apposition were less pronounced with SRμCT. Using scanning nanoindentation, both the indentation modulus and the hardness of the newly formed bone were highest in the E-Ti/CS, G-Ti/CS and G-Ti groups as compared to C-Ti ($P < 0.05$). Coatings with collagen and CS appear to improve both the quantity and quality of bone formed around Ti implants in ovarectomized rats. A simultaneous ethinyl estradiol- and genistein-rich diet seems to enhance these effects.

1. Introduction

Postmenopausal osteoporosis is a highly relevant disease, caused by naturally or surgically induced restriction of the ovarian function, resulting in estrogen deficiency, pronounced bone loss, increased fracture risk, severe accessory symptoms and high social and financial burdens [1]. In patients with such a compromised bone metabolism, aseptic loosening of metallic implants remains an issue of concern in trauma, orthopedic and maxillofacial surgery because osseointegration of Ti implants is impaired in osteoporotic bone [2–4]. HRT is the standard treatment of post-menopausal, estrogen-dependent symptoms of old age. HRT is also known to positively effect bone mass and fracture risk. But long-term HRT may bear substantial health risks, as indicated by the Woman's Health Initiative (WHI) and other clinical studies [5,6]. In this context alternative therapies based on the nutritionally relevant phytoestrogens became the focus of attention. Isoflavone such as genistein are promising candidates to replace HRT due to their

* Corresponding author at: Institute of Immunology, Medical Faculty "Carl Gustav Carus", Technische Universität Dresden, Fetscherstrasse 74, Dresden 01307, Germany. Tel.: +49 3514586528; fax: +49 3514586316.
E-mail address: Jan.Dudeck@tu-dresden.de (J. Dudeck).

http://dx.doi.org/10.1016/j.actbio.2014.01.034
1742-7061/

structural and functional similarities with the endocrine estradiol [7]. But the relevance of phytoestrogens in preventing osteoporotic fractures and implant loosening is still insufficiently studied. The increasing number of elderly patients suffering osteoporotic fractures leads to a higher demand for surgical techniques and the need for implants with improved osteogenic properties. One method of improving osseointegration is implant coating with inorganic [8] and organic [9] components of the physiological extracellular bone matrix (aECM). Several preclinical studies have investigated the in vivo effects of implant coating with type I collagen [10,11]. Other promising components of the ECM are glycosaminoglycans (GAGs) such as chondroitin sulfate (CS). Coating titanium implants with collagen and CS resulted in a significantly increased bone implant contact (BIC) around intramedullary Ti pins in the tibiae of healthy rats [12] and around dental implants in the mandible of healthy minipigs [13] when compared to uncoated implants. Similar results were shown in studies using CS as an additive in calcium-phosphate-based bone substitutes in small and large animal models [14]. However, in all these studies, healthy subjects with intact bone metabolism were used, hardly reflecting the situation in elderly, osteoporotic patients. Remarkably, the effect of collagen and CS on osseointegration of Ti implants under estrogen deficiency has not yet been investigated.

GAGs are ubiquitous components of the ECM and cell membrane in all eukaryotic organisms bridging extracellular stimuli and intracellular signalling. GAGs are negatively charged, linear polysaccharide chains composed of repeating disaccharide units which carry different amounts of sulfate groups at varying positions. Most of the GAGs have a molecular weight ranging between 10 and 100 kDa and are commonly present as components of proteoglycans (PGs) in various tissues [15]. CS consists of N-acetylgalactosamine and uronic acid. It is the most abundant GAG in cartilage, tendons, ligaments, brain and cancellous and cortical bone. In bone and cartilage CS mostly exists as a component of the PGs decorin, biglycan and aggrecan [16]. Furthermore, CS is abundantly present in mineralized cartilage but mostly restricted to calcified nodules and to the surface of osteocytes, osteocyte lacunae and canaliculi in mineralized bone in rats [17]. Among other GAGs, CS is able to bind cytokines and growth factors involved in bone regeneration [18,19]. Osteoblast attachment, collagen deposition and matrix mineralization have been reported to be directly dependent on GAGs [20,21]. Furthermore, sulfated GAGs are capable of binding calcium and calcium phosphates, including hydroxyapatite [17,22]. Recently, it has been suggested that GAGs also play a functional role in osteoclastogenesis [23]. These functional heterogeneity differences might be related to the dosage, type, molecular weight and sulfatation of GAGs, influencing their capability to bind cytokines and growth factors as well as to interact with cells and ECM components [24,25]. However, the molecular mechanisms leading to these effects are not completely understood.

Anchorage and stability of endosseous implants depend on the quantity and quality of peri-implant bone formation [26–28]. Methods such as synchrotron-radiation micro-computed tomography (SRμCT) and nanoindentation provide information about both the bone quantity and quality at the micro-scale, making them beneficial in assessing osseointegration [29,30]. Conventional μCT systems use polychromatic X-rays that cause image artifacts by beam hardening and interface scattering, especially at the implant–bone interface, resulting in a detection of higher absorbing species up to a distance of 200 μm around metal implants that are not present in reality [31]. This impairs the quantification of peri-implant bone structures and leads to a prominent overestimation of bone around metal implants. Therefore the use of standard μCT is limited for the evaluation of metal osseointegration [32]. A key benefit of SRμCT is the elimination of such image artifacts due to the high primary X-ray photon flux of the synchrotron radiation that enables the use of intensive, monochromatic X-rays. SRμCT provides therefore more sensitive detection of bone close to metal implants than standard μCT and a reliable quantification of peri-implant bone up to a distance of ~18 μm to the implant surface. Only directly at the implant–bone interface does SRμCT show a difference of ~10% in newly formed bone (BIA) as compared with histology, which may be attributed to the partial volume effect [31]. Nanoindentation enables spatially resolved measurements of mechanical properties such as Young's modulus and hardness at the micro-scale [33]. Due to the strong correlation between the micromechanical properties and both the bone mineral density and constitution of the organic bone matrix [34,35] this technique allows for conclusions regarding the peri-implant bone quality and the potential stability of implant anchorage [26].

The quality of bone formed around aECM modified Ti implants has not yet been investigated. The present study was therefore performed to evaluate the effects of CS-coated Ti implants on the peri-implant bone quantity and quality using SRμCT, nanoindentation, histomorphometry and histology. The impact of CS coatings on the osseointegration under estrogen deficiency was investigated using ovarectomized rats as an osteoporosis model. To test for the effects of an HRT on the osseointegration of CS-coated Ti implants under estrogen deficiency, an ethinyl-estradiol-rich diet was used to simulate a clinical relevant HRT. Furthermore, a genistein-rich diet was used to evaluate an alternative therapy based on the nutritionally relevant phytoestrogens.

2. Materials and methods

2.1. Implant preparation

Titanium (Ti6Al4V) pins of 0.8 mm diameter were used for the immobilization of various ECM components as described previously [12]. Briefly, the pins were cleaned with 1% triton X-100, acetone and 96% ethanol, rinsed in distilled water and dried. Type I collagen was obtained from bovine skin (IBFB Pharma GmbH, Leipzig, Germany) and dissolved in 10 mM acetic acid. Fibrillogenesis was allowed to take place overnight under physiological conditions (37 °C, pH 7.4 in 60 mM sodium phosphate buffer) in the presence of 10 mass% chondroitin-4-sulfate from bovine trachea (Sigma-Aldrich, Steinheim, Germany). Fibrils were collected by centrifugation and suspended. The Ti pins were incubated in the suspension at 25 °C for 1 min and air dried. The collagen and CS concentration adsorbed at the titanium implant was quantified using the Sirius red and Pieper method as recently described [36,37]. The final collagen amount on the implant surface ranged between 15 and 30 mg cm^{-2}. The properties of collagen type I and CS immobilized on titanium surfaces have been described in detail previously [36,37]. Uncoated Ti pins served as control implants. The coated implants were sterilized with gamma irradiation of 25 kGy, uncoated pins with ethylene dioxide. We found in preliminary studies that the degradation of lyophilized GAGs with molecular masses between 30 and 50 kDa (as used in this study) is minimal under gamma irradiation of 25 kGy in comparison to those of higher molecular weight (unpublished data).

2.2. Animal housing and diet

All animal handling and experimental conditions were licensed by the local animal care committee and carried out according to the Institutional Animal Care and Use Committee guidelines as regulated by the federal law governing animal welfare. 30 adult female Wistar rats (Charles River Laboratories, Sulzfeld, Germany) with a mean BW of 250–300 g were housed under controlled