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The effects of implant topography on osseointegration under estrogen deficiency induced osteoporotic conditions: Histomorphometric, transcriptional and ultrastructural analysis

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ABSTRACT

Compromised bone quality and/or healing in osteoporosis are recognised risk factors for impaired dental implant osseointegration. This study examined the effects of (1) experimentally induced osteoporosis on titanium implant osseointegration and (2) the effect of modified implant surface topography on osseointegration under osteoporosis-like conditions. Machined and micro-roughened surface implants were placed into the maxillary first molar root socket of 64 ovariectomised and sham-operated Sprague-Dawley rats. Subsequent histological and SEM observations showed tissue maturation on the micro-rough surfaced implants in ovariectomised animals as early as 3 days post-implantation. The degree of osseointegration was also significantly higher around the micro-rough implants in ovariectomised animals after 14 days of healing although by day 28, similar levels of osseointegration were found for all test groups. The micro-rough implants significantly increased the early (day 3) gene expression of alkaline phosphatase, osteocalcin, receptor activator of nuclear factor kappa-B ligand and dentin matrix protein 1 in implant adherent cells. By day 7, the expression of inflammatory genes decreased while the expression of the osteogenic markers increased further although there were few statistically significant differences between the micro-rough and machined surfaces. Osteocyte morphology was also affected by estrogen deficiency with the size of the cells being reduced in trabecular bone. In conclusion, estrogen deficiency induced osteoporotic conditions negatively influenced the early osseointegration of machined implants while micro-rough implants compensated for these deleterious effects by enhancing osteogenic cell differentiation on the implant surface.

Statement of Significance

Lower bone density, poor bone quality and osseous microstructural changes are all features characteristic of osteoporosis that may impair the osseointegration of dental implants. Using a clinically relevant trabecular bone model in the rat maxilla, we demonstrated histologically that the negative effects of surgically-induced osteoporosis on osseointegration could be ameliorated by the biomaterial's surface topography. Furthermore, gene expression analysis suggests this may be a result of enhanced osteogenic cell differentiation on the implant surface.

1. Introduction

Osteoporosis is a chronic disease affecting over 200 million people worldwide [1]. Osteoporosis in the elderly, especially postmenopausal women is also significantly correlated with tooth loss [2–5]. Lower bone density, poor bone quality and osseous microstructural changes, all characteristics of osteoporosis, have been shown to delay the bone healing process of fractured bone [6–8]. Endosseous dental implant healing exhibits similar biological mechanisms to that observed in bone fracture healing [9–12], and hence may be influenced by osteoporotic conditions. The results of a recent systematic review show that osteoporotic patients have higher rates of dental implant loss [13]. Many animal studies have reported a lower rate of titanium implant osseointegration in osteoporotic environments, however the majority of these studies used long bone [14–18] rather than jaw

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bone models [19]. As there are significant differences in the embryological origin, ossification process and the response to osteoporotic conditions between long bones and craniofacial bones [20,21], the relevance of results obtained in a long bone model to the oral environment is questionable. We have shown that the bone quality in the posterior rat maxilla is negatively affected by estrogen deficiency induced osteoporotic conditions [22], and that the first molar site in the posterior maxilla is a suitable model for dental implant research [23].

Early studies in the jawbone using an osteoporotic rat model showed no significant influence on osseointegration when using first-generation 'machined' implants [24–26]. However, contemporary implants have a 'micro-rough' implant topography which is known to influence peri-implant bone healing [27]. Indeed animal studies have shown that commercially available 'micro-rough' surfaced titanium implants result in superior bone to implant contact compared to 'smoother' machined surfaced implants [28,29], as well as having superior torque removal values [30,31]. Few studies have evaluated the influence of titanium implant surface topography on the early stages of bone healing during osseointegration under osteoporotic conditions [32,33]. Furthermore, the underlying cellular and molecular mechanisms that may be influenced by surface topography during osseointegration under osteoporotic conditions are not well understood.

Therefore, in this study, the primary aim was to test the hypothesis that estrogen deficiency has a negative influence on implant healing which can be ameliorated by micro-rough implant surface topography. A secondary objective was to undertake ultrastructural and gene expression analysis to elucidate the cellular and molecular mechanisms that may be influenced under these conditions.

2. Material and methods

2.1. Animals

The Griffith University animal ethics committee approved the experimental protocol for the study (DOH/01/4/AEC). Sixty-four three-month-old female Sprague-Dawley rats (Animal Resource Center, Western Australia) were used. This number of animals was chosen based on the results of histomorphometric analysis in similar studies using ovariectomised rats [15,23]. Animals were fed standard rat chow and water ad libitum throughout the experiment. After acclimatization for 2 weeks, the rats were randomly divided into two groups, sham-operated (SHAM, n = 32) and ovariectomised (OVX, n = 32). Ovariectomy was performed according to our previously established methods [15,22] where both histological and micro-CT analyses demonstrated successful induction of osteoporosis. SHAM group rats were also subjected to the same surgical procedure with an equivalent amount of fat tissue removed instead of the ovaries. All of the surgical procedures were performed under isoflurane (1–3%) inhalation anaesthesia. The animals were subsequently allowed to develop osteoporosis over three months prior to implant placement. This period of time has been shown to be sufficient to develop osteoporotic conditions in this model [22].

2.2. Implants

The surface roughness parameter 'Sa' (Arithmetic mean height) was analysed under 20 × objective magnification using 3D optical microscopy (Contour Elite 3D, Bruker, US). 'Minimally-rough' machined (Sa = 518.7 ± 10.88 nm; Sq = 643.4 ± 30.53 nm) and 'micro-rough' (Sa = 906.19 ± 19.85 nm; Sq = 1.11 ± 0.09 μm) surfaced titanium implants (2 mm diameter × 3 mm length) produced from Type IV commercially pure titanium were obtained from Southern Implants Ltd (Irene, South Africa). The micro-rough surfaced implant was prepared using the same techniques (aluminium oxide blasting) as used for commercially available dental implants (Fig. 1).

2.3. Surgical procedures

One implant of each surface type (machined and micro-rough) was placed bilaterally in the maxilla of all 64 animals 3 months

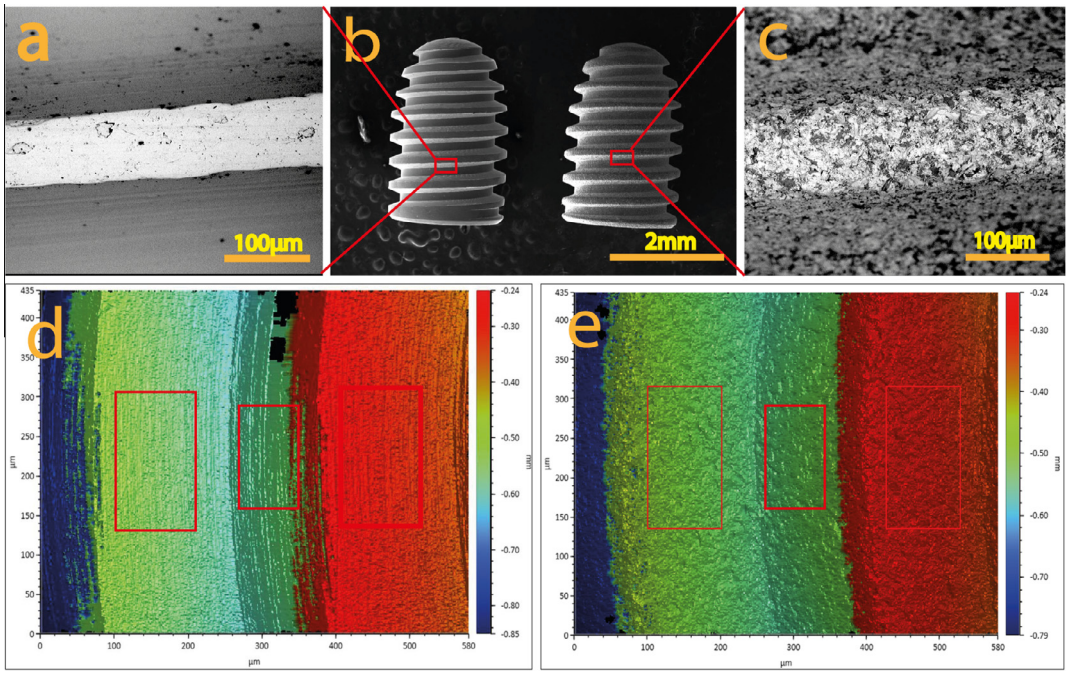

Fig. 1. Machined (a, b) and micro-rough (b, c) surfaced implants viewed under SEM. The roughness (Sa) of the implants in both implant thread valley and apex areas (red rectangles) as measured by 3D optical microscopy were 518.7 ± 15.38 nm for the machined surface implant (d) and 906.19 ± 28.07 nm for the micro-rough surfaced implant (e). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)