Acta Biomaterialia 39 (2016) 169–179

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Early bone anchorage to micro- and nano-topographically complex implant surfaces in hyperglycemia

Elnaz Ajami [a], Spencer Bell [a], Robert S. Liddell [b], John E. Davies [a,b,*]

[a] Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada
[b] Faculty of Dentistry, University of Toronto, Toronto, ON, Canada

ARTICLE INFO

Article history:
Received 4 February 2016
Received in revised form 20 April 2016
Accepted 11 May 2016
Available online 12 May 2016

Keywords:
Diabetes mellitus
Hyperglycemia
Dental implants
Osseointegration
Nanotopography
Interfibrillar and intrafibrillar collagen mineralization

ABSTRACT

The aim of this work was to investigate the effect of implant surface design on early bone anchorage in the presence of hyperglycemia. 108 Wistar rats were separated into euglycemic (EG) controls and STZ-treated hyperglycemic (HG) groups, and received bilateral femoral custom rectangular implants of two surface topographies: grit blasted (GB) and grit-blast with a superimposed calcium phosphate nanotopography (GB-DCD). The peri-implant bone was subjected to a tensile disruption test 5, 7, and 9 days post-operatively (n = 28/time point); the force was measured; and the residual peri-implant bone was observed by scanning electron microscopy (SEM). Disruption forces at 5 days were not significantly different from zero for the GB implants ($p = 0.24$) in either metabolic group; but were for GB+DCD implants in both metabolic groups ($p < 0.001$). Contact osteogenesis was greater on GB-DCD than the GB surface. The nano-and micro-surfaced implants showed significantly different disruption forces at all time points (e.g. >15 N and <5 N respectively at 9 days). Such differences were not seen within the GB implants, as all values were very low (<5 N). Even in hyperglycemia the GB-DCD surface outperformed the GB surfaces in both metabolic groups. Significantly, SEM of peri-implant bone showed compromised intra-fibrillar collagen mineralization in hyperglycemia, while inter-fibrillar and cement line mineralization remained unaffected. Enhanced bone anchorage to the implant surfaces was observed on the nanotopographically complex surface independent of metabolic group. The compromised intra-fibrillar mineralization observed provides a mechanism by which early bone mineralization is affected in hyperglycemia.

Statement of Significance

It is generally accepted that the hyperglycemia associated with diabetes mellitus compromises bone quality, although the mechanism by which this occurs is unknown. Uncontrolled hyperglycemia is therefore a contra-indication for bone implant placement. It is also known that nano-topographically complex implant surfaces accelerate early peri-implant healing. In this report we show that, in our experimental model, nano-topographically complex surfaces can mitigate the compromised bone healing seen in hyperglycemia. Importantly, we also provide a mechanistic explanation for compromised bone quality in hyperglycemia. We show that intra-fibrillar collagen mineralization is compromised in hyperglycemia, but that interfibrillar and cement line mineralization, remain unaffected.

1. Introduction

Chronic hyperglycemia, pathognomonic of diabetes mellitus, is known to have a significant impact on bone healing. Nevertheless, the increasing success of dental implants, along with the realized benefits of implant therapy, have accommodated patients with controlled diabetes (i.e. patients diagnosed with diabetes, and who undertake various strategies to control their blood glucose levels) as candidates for treatment [1–3]. However, for those who fail to control their glucose levels, the attendant uncontrolled hyperglycemia is considered a contra-indication for dental implant placement. In 2012, from an estimated total of 29.1 million Americans suffering from diabetes, only 21 million were formally diagnosed, leaving approximately 8.1 million people — which was over 3% of the total US population in 2012 — undiagnosed and therefore unaware of their hyperglycemic condition [4]. While

* Corresponding author at: Institute of Biomaterials and Biomedical Engineering, University of Toronto, 164 College Street, Toronto, Ontario M5S 3G9, Canada.
E-mail address: davies@ecf.utoronto.ca (J.E. Davies).

http://dx.doi.org/10.1016/j.actbio.2016.05.017
1742-7061/

survival rates of endosseous root-form dental implants range from 85% for fixed prosthodontics to 95% and higher for single implants [5], undiagnosed hyperglycemia could be a potential contributor to the remaining 5% of implant failures that occur clinically for unknown reasons.

Despite the controversies in the literature [6], it is generally agreed that diabetic bone is weaker and more fragile than healthy bone [7,8], but the reasons remain unknown. Diabetic individuals have higher rates of osteoporotic fractures [9], decreased skeletal growth during adolescence [10], and delayed or impaired fracture healing [11]. Reports of decreased mechanical retention of implants [12] and bone implant contact (BIC) in type I diabetes [13–15] lend additional support to the notion of compromised peri-implant healing in diabetic subjects. Indeed, reduced osteoid formation, delayed osteoid mineralization, and reduced overall bone volume and maturation in hyperglycemia were reported by Goodman & Hori [16]. But while the effects of hyperglycemia on bone healing have been addressed from a few weeks to several months, little has been done to elucidate its effects on the earliest stages of the bone healing cascade. Yet, it is generally believed that early healing events govern the long-term success of endosseous implants. Indeed, we have recently shown that bone remodeling is delayed in hyperglycemic rats by about 5 days, as compared to a euglycemic population, and there were striking differences in reparative bone volume between the two metabolic groups [17]. Such adverse effects at early stages of healing could negatively impact long-term implant stability, and thus contribute to implant failure.

It is also widely accepted, with the emergence over the last decades of topographically complex implant surface designs, that clinical success rates are improved compared to less topographically complex machined surfaced implants, as shown experimentally by Buser et al. [18], particularly in Class III and IV (as defined by Lekholm and Zarb [19]). We have recently described the biological significance of three different scale-ranges of implant surface topography (sub-micron, micron and coarse-micron) in terms of both the "true" and "functional" bone/implant interface [20], and that these mimic the topographic scales ranges of bone at natural remodeling sites [21]. In this context therefore, more topographically complex surfaces will exhibit features at all scale ranges compared to less complex surfaces. Thus, the possibility arises that topographically complex implant surface designs could mitigate the impaired peri-implant bone healing associated with hyperglycemia. We have recently shown that higher bone/implant contact (BIC) values are observed on nanotopographically complex surfaces in hyperglycemic subjects when compared to microtopographically complex implant surfaces in euglycemic subjects [17]. BIC is the histological outcome of the combination of osteoconduction and bone formation. We have defined osteoconduction as the recruitment and migration of osteogenic cells to the implant surface [22] — and shown that this is increased on nanotopographically complex implant surfaces in both euglycemia and hyperglycemia [17,23]. Such contact osteogenesis is an essential pre-requisite for bone anchorage to the implant surface, and while these mechanisms have been explained in more detail elsewhere in healthy animals [24,20], we have not assessed the effects of hyperglycemia on bone/implant anchorage.

Thus we sought, herein, to investigate the temporal effects of hyperglycemia on early bone anchorage to candidate implant surfaces up to 9 days post-implantation in rats. Specifically, we asked whether hyperglycemia would negatively impact bone/implant interfacial stability at such early stages of healing; and whether such impaired bone anchorage could be mitigated by the addition of nanotopographical complexity to a microtopographically complex implant surface. To address this experimentally, we inserted custom implants of two different surface topographies in the femora of either euglycemic or hyperglycemic rats and employed a mechanical disruption methodology which we have described in detail elsewhere [25] to assess bone anchorage. We hypothesized that bone/implant anchorage on the nanotopographic surface in hyperglycemia would outperform that seen on the implant surfaces absent of nano features in euglycemia.

2. Materials and methods

2.1. Candidate implant samples

Custom rectangular implants (4 mm × 2.5 mm × 1.3 mm) were kindly fabricated by BIOMET*3i* (FL, USA) from commercially pure Grade 4 titanium. Each implant had a central 0.7 mm hole drilled down the long axis to enable suture fixation at surgery, and, later, to facilitate mechanical testing. Two groups of implants were prepared. All underwent a grit blast treatment to create a microtopographically complex surface (GB). The second group was further treated with calcium phosphate nanoparticles (hydroxyapatite; 10–100 nm) to create a nanotopographically complex surface (GB-DCD). These two surfaces are described as the micro- and nano-surfaces, respectively.

2.2. Surgical procedure

108 male Wistar rats were used in this experiment. All experimental protocols were approved by the Ethics Committee of Animal Research at the University of Toronto. Hyperglycemia was induced via a single intravenous injection of streptozotocin (STZ; 65 mg/kg) 1 week pre-operative. Control animals received an equivalent injection of saline. A blood glucose level of >15 mmol/L in the first 48 h (and maintained thereafter) was considered hyperglycemic for the treated group, and animals underwent surgery 1 week post-induction. To measure blood glucose, following standard tail vein puncture, a drop of blood was collected in a glucose test strip (FreeStyle Lite, Abbott Diabetes Care Inc., Alameda, CA) which was then loaded into a conventional glucometer (FreeStyle Lite). In this way only the bone formed during the hyperglycemic period would have been subject to the influence of the new glycemic level – and we have previously shown that such bone can be identified [17]. Rat chow was provided ad libitum and all animals were allowed free access to water throughout the study. Implants were placed bilaterally into the distal femora of rats as described previously [26]. Briefly, the antero-lateral aspect of one hind limb was shaved. Incisions were made in the skin and underlying muscle to expose the femur. A 1.3 mm twist drill (Brasseler, GA, USA) attached to a dental handpiece (ImplantMED DU 900 and WS-75, W&H, Dentalwerk, Austria) was used to create a bicortical hole in the bone, approximately 4 mm (depth) × 1.3 mm (diameter), perpendicular to the long axis of the femur. A pair of dividers was calibrated to guide a second bicortical hole, parallel and approximately 2.5 mm distant from the first one. Following this, a 1.2 mm cylindrical side-cutting bur (Biomet 3i, FL, USA) was used to join the holes, in a proximal–distal direction. Irrigation with saline was used throughout the preparation. An implant was then press-fit into the defect and a suture (4-0 PolysorbTM, Syneture, USA) was used to keep it in position during the post-operative period by threading through the central hole and around the lateral femoral margin. Muscle tissue was sutured with the same biodegradable sutures, and the cutaneous tissues were re-apposed using staples (9 mm wound clips, Becton Dickinson, MD, USA). This processs was then repeated on the contralateral femur. Each animal received both a GB and a GB-DCD implant; the side of implantation, right or left femur, was assigned by partial randomization.