Bone Healing and the Effect of Implant Surface Topography on Osteoconduction in an Environment of Uncontrolled Hyperglycemia

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Introduction:

Uncontrolled diabetes is a contra-indication for dental implant placement, yet there are about 7 million undiagnosed hyperglycemic diabetics in North America alone [1]. Such undiagnosed systemic conditions may be related to the 5% of dental implants that fail clinically for unknown reasons. Reported findings related to peri-implant bone formation and remodeling in hyperglycemia are contradictory, although the general consensus is that hyperglycemia does compromise bone growth [2].

In the present study, we sought to monitor the effects of hyperglycemia on early bone healing and, specifically, to test the effect of hyperglycemia on osteoconduction, in the presence of candidate implant surfaces, using a model we have previously described [3]. Thus, we hypothesized that hyperglycemia would delay early bone healing by impeding osteoconduction. Since osteoconduction, together with bone formation, results in contact osteogenesis; and since nano-topographically complex implant surfaces have previously been shown to accelerate osteoconduction [3], we also hypothesized that comprised implant integration wrought by hyperglycemia could be abrogated by using nanotopographically complex endosseous implants.

To address our hypotheses we undertook two parallel experiments. In the first we created femoral osteotomies in both hyperglycemic and healthy rats and tracked temporal bone healing by MicroCT analysis. In the second, we measured bone-implant contact, in both hyperglycemic and healthy rats using custom bone ingrowth chambers modified with either micro- or nano-topographically complex surfaces.

Materials and Methods:

The experimental protocols were approved by the local animal care committee of the Faculty of Dentistry, University of Toronto. In both cases hyperglycemia was induced by intraperitoneal injection of streptozotocin (65mg/kg) one week prior to surgeries (HG group); controls (HC) were injected with the same volume of sterile saline. Eighty animals were used for the osteotomy wound model (20 of these animals received fluorochrome labels on the 7th post-operative day and at 7 day intervals thereafter for 30 days) and 100 animals for implant placement. The final animal groups consisted of 4 groups: 40 HC and 40 HG (osteotomy); 50 HC and 50 HG (implant placement).

Osteotomy: Bilateral mono-cortical drill hole defects were created in the distal femora of rats. Samples were harvested at 5, 10, 15 and 30 days (n=10 per time point). Samples harvested at 5, 10 and 15 days were scanned using a Scanco MicroCT40 (Basserdorf, Switzerland) at 70 kVp and 114 μA with resolution of 6 μm in 3 planes. Bone volume per total volume (BV/TV %), connectivity density and trabecular thickness and number were measured within the marrow space. Selected samples harvested at 30 days were resin embedded for fluorescence microscopy.

Implant Placement: 200 implant chambers with internal dimensions of 3x3x1mm (Biomet 3i; FL, USA) with 4 different internal surface topographies [dual acid etch (DAE), DAE + calcium phosphate nanocrystals (DCD), DAE + nanotitanate (NAT) and DAE + micro acid etch (MAE)] were implanted bilaterally in rat femora. All rats were euthanized at 9 days (n=25 per surface type) to

compare with our previously published data. All harvested samples were resin embedded for multiple block face microscopy and backscattering electron imaging. BIC was measured using image analysis software. For statistical analysis, a representative median value of BIC% was calculated. The data was evaluated based on the %probability of each individual value (BIC% for the whole chamber) to fall above/below the median.

Results:

Post-operative healing was uneventful; but 3 "osteotomy" rats failed to become hyperglycemic and were excluded from the study. Similarly, 5 femora in the implant groups were found to be fractured at harvest, and also excluded from the study.

MicroCT analysis showed significantly less BV/TV%, connectivity density and trabecular number in the HG group compared to the HC group at 5 days, with no statistical differences at 10 and 15 days, except the differences in connectivity density at 10 days. Between 5 and 10 days, BV/TV% and number of trabeculae increased in the HG group, while they decreased in the HC group. Both BV/TV% and trabecular number in HG group at 10 days were not significantly different from those in HC group at 5 days. BV/TV%, connectivity density and trabecular number decreased within metabolic groups from 10 to 15 days, and the rate of decrease was similar between metabolic groups. Fluorescence images obtained, under the same acquisition parameters, showed that the HG group exhibited considerably less fluorescent intensity compared to HC samples.

In the implant groups, no statistical differences were observed between HG and HC for any one of the surface types, but HG showed less mean BIC% compared to HC for all surfaces. BIC% comparisons revealed statistical significances between surfaces and between metabolic groups. Comparisons within metabolic groups between surfaces showed significantly higher distribution of BIC% values above the median for the NANO surfaces compared to the MICRO surfaces, except for NAT-HG vs. DAE-HG. Comparisons between metabolic groups and between surfaces indicated a higher distribution of %BIC values above the median for NANO surfaces in HG group compared to MICRO surfaces in HC group. However, the statistical differences were only observed between DCD-HG& DAE-HC, DCD-HG&MAE-HC, and NAT-HG&MAE-HC.

Discussion:

Healing of rat femoral diaphyseal osteotomies proceed (following blood clotting) by florid reparative trabecular formation, cortical closure, and remodelling of the reparative medullary trabeculae to restore homeostasis. Our microCT data provide clear evidence that bone healing is delayed in an environment of hyperglycemia in this model. This means that in HG animals the reparative trabecular bone formation occurs later as is remodelling when compared to healthy controls. Thus, the reparative bone volume changes with time in the following sequence: HC>HG 5 days; HG>HC 10 days; HG>HC 15 days. However, the fluorochrome labelling would suggest that the quality/density of the HG bone is also compromised compared to that of the HC groups. Clearly, with these temporal changes, some of the conflicting data in the literature can be explained, since depending upon the duration of a study a HG animal may display either less, or more, bone than an HC animal. However, it also illustrates that the single time-point chosen for our implant study was less than ideal, both because it was not a temporal study and because the time-point is close to that at which the HG animals have more reparative bone than the controls. Nevertheless, it was clear that contact osteogenesis on NANO surfaces exceeded that seen on MICRO surfaces in both HC and HG groups, and NANO surfaces in hyperglycemic animals outperform MICRO surfaces in normal animals. These results demonstrate the importance of tracking temporal changes when elucidating the effects of hyperglycemia on peri-implant bone healing.

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