The Comparative Performance of Micro- and Nano-topographically Complex Endosseous Implant Surfaces in Normoglycemic and Hyperglycemic Subjects

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Introduction:

Endosseous implants are extremely effective with a clinical success rate of 95%.[1] There are numerous factors that contribute to the failure of the remaining 5% of implants, of which recent dental literature suggests undiagnosed systemic diseases such as diabetes mellitus, and more specifically the associated hyperglycemic environment, which causes a delay in early stages of bone healing, to be a possible contributor. In 2011, an estimated 7 million Americans suffered from diabetes yet remained undiagnosed.[2] As chronic hyperglycemia can exist for many years before a diagnosis is made, developing endosseous implants capable of overcoming the delayed bone healing associated with hyperglycemia may be of paramount importance for improving clinical success rates.

Recently engineered nanotopographically complex implants have been shown to increase *osteoconduction*, defined as "the recruitment and migration of osteogenic cells through a 3-dimensional, transient, soft biological matrix", during early time points in a healthy population, resulting in increased *de novo* bone formation on the implant surface thereby improving implant stability and decreasing healing times.[3] The success of such surface designs has received attention in recent years, yet their modulating effect on bone healing has yet to be investigated in environments of compromised healing.

The aim of this project was to investigate nano-surfaces and their modulating effect on bone healing in an animal model of hyperglycemia. It was hypothesized that, although hyperglycemia was expected to delay osteoconduction on both micro- and nanotopographically complex surfaces, osteoconduction on a nanotopographically complex surface in an environment of uncontrolled hyperglycemia would be greater than on a microtopographically complex surface in normoglycemic conditions.

Materials and Methods:

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. Successful induction of hyperglycemia was defined as a blood glucose level greater than 15mmol at 48 hours post-injection. In total, 124 male Wistar rats were used.

Custom rectangular implants (Biomet 3i, Florida, USA) were made from commercially pure titanium (dimensions: 4x2.5x1.3mm (LxWxH) with a 0.7mm diameter hole down the long axis). All implants underwent a standard grit blasting treatment to create a microtopographically complex surface. Half of the implants were then further treated with calcium phosphate nanoparticles to create a nanotopographically complex surface (DCD). These two surfaces were used as the micro- (GB) and nano-surfaces (GB-DCD), respectively. Implants were placed bilaterally in the distal femora of the rats, with each receiving both a GB and a GB-DCD implant. The side of implantation was assigned by partial randomization. Samples were harvested at 2- and 4-days (n=8 rats per time point), and 5-, 7-, and 9-days (n=36 rats per time point) post-operative. Samples from each time point (n=8) were collected for histology and BSEI. Mechanical testing was conducted on samples from 5-, 7-, and 9-days (n=28) to assess stability at the bone-implant interface using a newly

designed tensile model adapted from previous work.[4] Implant surfaces were qualitatively analyzed following mechanical testing to assess the fracture planes. A non-parametric Kruskal-Wallis test followed by post-hoc Wilcoxon rank sums tests for multiple comparisons was conducted to analyze mechanical testing groups. Statistical significance was set at $p<0.01$.

Results:

Disruption force values were significantly greater in the Control group than the HG group with the nano-surface at 7 days ($p<0.0001$) and 9 days ($p=0.0042$), while no difference was detected at 5 days. Performance was significantly greater in the nano-surface compared to the micro-surface at 5 days (Control $p<0.0001$; HG $p<0.0001$), 7 days (Control $p<0.0001$; HG $p<0.0001$) and 9 days (Control $p<0.0001$; HG $p<0.0001$). Furthermore, the GB-DCD surface in the HG group performed significantly better than the GB surface in Control animals at 5 days ($p<0.0001$), 7 days ($p<0.0001$) and 9 days ($p<0.0001$). There was a considerable increase in performance after only 5 days of healing with the nano-surface compared to the micro-surface in both the control and HG groups (1384% and 799%, respectively), which was not expected. Comparing the GB-DCD surface at successive time points in both metabolic groups, there was a significant increase in disruption force values from 5 days to 7 days (Control $p<0.0001$; HG $p<0.0001$) and 7 days to 9 days (Control $p=0.002$; HG $p=0.0004$). This trend was not matched by the GB surface.

Residual bone on implant surfaces following mechanical testing showed substantially more bone on nano-surfaces at all time points, confirming trends seen in the mechanical testing data. More importantly, bone on the GB-DCD surfaces fractured further from the implant surface, leaving visibly larger and thicker sections of residual bone, indicating peri-implant bone of greater maturity. Residual bone on the nano-surface was considerably greater than the GB surface at 5 days, which was unexpected and matched the trend in mechanical testing data. There was little visual difference among the GB surface images. BSEI clearly showed a delayed onset of fracture healing, as well as compromised mineralization, in the peri-implant bone of hyperglycemic rats.

Discussion:

Overall, it was evident that hyperglycemia caused a delayed response in the early stages of the peri-implant endosseous healing response. Mechanical testing data showed a decreased performance in the presence of hyperglycemia, while BSEI clearly showed a delay in the early stages of healing in addition to compromised bone quality in the peri-implant region. These findings corroborate previous results and suggest a contraindication for the success of implant-based therapies. Our results showed a significantly greater performance in mechanical testing when using the nano-surface, regardless of time or metabolic status of the animal. Nowhere was this more evident than at 5 days, which showed a substantial increase in performance with the nano-surface. As an increase in mechanical testing data is correlated to an increased rate of osteoconduction and *de novo* bone formation at the surface, the existence of a difference at such an early time point indicates accelerated bone formation on the nano-surface, since, without bone formation, mechanical testing values would be zero. Furthermore, since implants differed only in their nanotopographical features, it could only be through the increased osteoconduction and acceleration of the early stages of the bone healing cascade, and the subsequent increase in *de novo* bone formation, that led to superior performance of the nano-surface. Thus, it was evident that contact osteogenesis was increased on nanotopographically complex surfaces, even in an environment of uncontrolled hyperglycemia, leading to increased bone maturity at earlier time points and greater early implant stability.

Acknowledgements: The authors would like to acknowledge NSERC and Biomet 3i for their generous financial support of this work.

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