Gene Expression of RAW264.7 Macrophages Grown on Smooth and Rough Implant Surfaces

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Introduction:

Osseointegration, the direct attachment of living bone to an implant surface, is necessary for the success of dental implant therapy. Surface topography of an implant influences cellular behaviour and osseointegration. Chehroudi *et al.* (2009) found that rough surfaces accumulate macrophage, which was subsequently associated with bone formation. Macrophages exhibit classic inflammatory (M1) or wound-healing (M2) phenotypes that secrete different cytokines, chemokines and growth factors. Our experiments aim to characterize the phenotypes of macrophages grown on polished and sandblasted and acid-etched (SLA) surfaces using gene expression microarray technology.

Materials and Methods:

Epoxy replicas of polished and SLA surfaces were made from titanium models following the highly accurate technique described by Schuler *et al. (*2009) to replicate features within in the submicrometer range. The replicas were cleaned and sterilized in argon plasma before being seeded with RAW264.7 murine macrophages. Cells were cultured for 1 and 5 days and RNA was isolated. RNAs with RNA integrity numbers (RINs) >8.0 were analyzed with a whole genome microarray (Agilent) and a protease microarray (CLIP-CHIP™). Data analysis with GeneSpring (Agilent) and IPA (Ingenuity Systems) identified top associated network functions. Qualitative polymerase chain reaction (qPCR) was used to confirm differences in gene expression.

Results:

Whole genome microarray analysis showed differential expression of 199 genes on day 1 and 4943 genes on day 5 on SLA surfaces. Differences in gene expression over time were apparent; 816 genes and 166 genes were differentially expressed on polished and SLA surfaces respectively. These genes were assigned to network categories related to cellular movement, immune cell trafficking and inflammation. Confirmation of expression of ADAMTS3, Cys C, HSP, MMP12, CCL4, CCL7, CTNND1 and ADM was performed with qPCR. Expression of CCL13 and C1qa was too low to analyse. While ADAMTS3, Cys C, HSP, CTNND1, and ADM did not exhibit detectable differences in gene expression between surfaces, MMP12 and CCL4 were significantly upregulated on SLA surfaces by factors of 2.39 and 2.18 after 1 day, and CCL7 was significantly upregulated on SLA surfaces by a factor of 4.01 after 5 days. On the protease CLIP-CHIP™, no significant difference in genes expression was found.

Discussion:

Whole genome analysis appears to be a useful tool for screening of gene expression on different surface topographies. Many genes associated with inflammation are upregulated on SLA surfaces. qPCR confirmed microarray results, showing upregulation of expression of MMP12 and CCL4 after 1 day, and CCL7 after 5 days. Further confirmation of expression of genes specific to M1 and M2 polarization with qPCR is in progress

References:

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