Whole-Blood Gene Profiling of Patients Fitted with a Percutaneous Osseointegrated Lower-Limb Prosthesis

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Introduction
An estimated 150,000 people in the United States undergo a lower-limb amputation each year with over 1,000,000 people currently living with one. The current standard of care for these patients is to provide a socket-based prosthetic which is associated with muscle atrophy, stump pain, altered gait, and back pain. A percutaneous osseointegrated prosthesis (POP) can alleviate many of these issues but little research has investigated the in-vivo healing process associated with implantation surgery and associated systemic markers of healing. In this study we analyzed gene expression at multiple time points from patients who were implanted with a POP.

Methods
Patients recruited to participate in an FDA-approved, Early Feasibility Study of a POP device, underwent a two-stage surgical procedure to be fitted with an osseointegrated implant with a wait time of 4-6 weeks between surgeries. Whole-blood samples were collected from these patients at the following time points: pre-surgery 1 (PrS1), post-surgery 1 (PoS1), pre-surgery 2 (PrS2), post-surgery 2 week 1 (PoS2-W1), post-surgery 2 week 2 (PoS2-W2), post-surgery 2 month 1 (PoS2-M1), post-surgery 2 month 3 (PoS2-M3), post-surgery 2 month 6 (PoS2-M6), post-surgery 2 month 9 (PoS2-M9), and post-surgery 2 month 12 (PoS2-M12). Whole-blood RNAs were isolated and single-end base pair sequencing was performed. Gene read counts were quantified via featureCounts and differential gene expression (DEG) analysis was performed using DESeq2. For the DEG analysis, pair-wise comparisons were made in two sets: first, between PrS1 and all subsequent time points; second, between PrS2 and all subsequent time points. Results from the DEG analysis were used to perform an enrichment analysis which implemented topGO’s elim graph algorithm paired with the Fisher’s test for significance. Enrichment analysis were run on each of the Gene Ontology’s (GO) sub ontologies: biological process (bp), cellular component (cc) and molecular function (mf).

Results
Compared to PrS1 baseline, the number of significant DEGs at each time point were: PoS1 (246), PrS2 (75), PoS2-W1 (51), PoS2-W2 (306), PoS2-M1 (109), PoS2-M3 (96), PoS2-M6 (27), PoS2-M9 (120), PoS2-M12 (94). Many non-coding (LINCs and LOCs) DEGs were statistically significant at all time points while DEGs related to hemoglobin were specific to PoS2-W1 and PoS2-W2. The number of DEGs identified when comparing to PrS2 baseline were: PoS2-W1 (171), PoS2-W2 (235), PoS2-M1 (182), PoS2-M3 (184), PoS2-M6 (186), PoS2-M9 (188), PoS2-M12 (169). Also, chemokine-related DEGs were identified at multiple time points. Compared to PrS1 baseline, significant GO terms were identified at the following time points for the GO sub ontologies (bp, cc, mf): PoS1 (319, 35, 39), PrS2 (178, 6, 24), PoS2-W1 (153, 22, 32), PoS2-W2 (158, 35, 35), PoS2-M1 (140, 33, 28), PoS2-M3 (135, 40, 51), PoS2-M6 (66, 13, 6), PoS2-M9 (246, 45, 56), PoS2-M12 (168, 41, 45). The number of significant terms when comparing to PrS2 baseline were as follows: PoS2-W1 (153, 22, 32), PoS2-W2 (158, 35, 35), PoS2-M1 (140, 33, 28), PoS2-M3 (135, 40, 51), PoS2-M6 (66, 13, 6), PoS2-M9 (246, 45, 56), PoS2-M12 (168, 41, 45). Similar to the differential expression analysis results, immune response related biological processes were identified at multiple time points.

Conclusion
Statistically significant DEGs were identified at all time point comparisons with gene expression primarily involved in biological processes related to immune response. PrS1 vs PoS2-W2 had the most DEGs with hemoglobin-related genes being unique to these time points and PoS2-W1. The presence of DEG PoS2-M12 imply that these patients exhibit a sustained response to osseointegrated implants. These results provide further insight into the biological processes involved in the post-surgery healing process of patients who receive POPs.