# Osteogenic Subset of LepR-expressing Cells Is the Origin of Fibrotic Tissue in a Mouse Model of Trans-femoral Percutaneous Osseointegrated Prosthesis Failure

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**INTRODUCTION:** Amputations are among the most common surgical procedures performed globally due to rising vascular and metabolic diseases, ongoing military conflicts and failure of limb-sparing procedures. For more than 2.1 million Americans living with limb loss, percutaneous osseointegrated prostheses (POPs) may have advantages over conventional sockets[1, 2]. The success of a POPs relies on the strong attachment of an intra-medullar implant to the amputated bone through osseointegration, similar to the attachment of an implant to bone in cementless total joint replacements (TJRs). Failure of this process to achieve effective initial osseointegration, often accompanying with peri-implant fibrosis, leads to aseptic loosening, a costly complication and one of the leading causes for the failure of POPs and TJRs[3,4]. Progress towards preventing these complications is crucial as current practice often requires revision surgery, which imposes additional morbidity and mortality risk on patients. Identifying the origin of the fibrogenic cells in peri-implant fibrosis will reveal targets for potential non-surgical therapies that can prevent peri-implant fibrosis or resolve an established fibrosis. Leptin receptor (LepR)-expressing mesenchymal stem cells can differentiate into progenitors of osteoblasts and adipocytes. In this abstract, we show that the osteogenic subset (ALPL+ ADIPOQ-) of LepR-expressing cells is the main origin of fibrotic tissue in peri-implant tissue in our mouse model of POP aseptic loosening.

**METHODS:** All experiments were approved by the IACUC.
Model of POP aseptic loosening: A circumferential skin incision with a posterior skin flap was made approximately 3mm below the knee joint. Femoral and sciatic nerves were resected, and major vessels were ligated or cauterized. The femoral shaft was cut transversely 1mm above the epicondyles and the distal extremity was removed. The intramedullary canal was retrogradely reamed to 50% larger than the diameter of the stem of a PMMA (Simplex HV) implant, resulting in a loose implant with potential micromotion and thus restricting osseointegration **(Figure 1.a)**. The implant was loosely inserted into the over-drilled canal, the quadriceps femoris muscle was sutured to the hamstring muscles, and the wound was closed.
Mice: Surgeries were performed on 10 to 13-week-old, male or female, LepR-Cre; TdTomato (n=5) or LepR-CreER; ZsGreen (n=3) mice to induce peri-implant fibrotic tissue. In the LepR-Cre mice, LepR-lineage cells (all the cells that had ever expressed LepR and their progeny) were marked with TdTomato. In the CreER mice, LepR-expressing cells (which were actively expressing LepR within ~1 week after tamoxifen injection) were marked with ZsGreen. Tamoxifen (2 mg, I.P.) was administered at day 5, 4, and 3 prior to implantation.
Histology and Immunofluorescence: Cryo-sectioned sections were stained with H&E for histological analysis or primary/ secondary antibodies for immunofluorescence.
Statistical Analysis: Data are reported as mean ± standard deviation. Two tailed student's T-test was used to compare groups. Significance was assigned to $p<0.05$.

**RESULTS:** At 2 weeks post-surgery, peri-implant lucency was observed in both radiograph and MicroCT images in all mice **(Figure 1.a and b)**. Histological analysis (H&E) showed remarkable fibrosis with abundant flat, elongated, and spindle-shaped cells in the peri-implant area **(Figure 1.c)**. Immunofluorescence analysis showed a significant amount of LepR-expressing cells **(Figure 2.a and b)** and LepR-lineage cells **(Figure 2.c and d)** in the peri-implant fibrotic tissue of the over-drilled POPs. Antibody staining showed a significant presence of ALPL expression in the fibrotic peri-implant LepR+ cells **(Figure 3.a)**. A significantly higher percentage of peri-implant fibrotic tissue was ALPL+ and did not express ADIPOQ **(Figure 3.d)**, confirming cell identity as from the osteogenic subset of LepR+ cells.

**DISCUSSION: Our mouse model can reproducibly induce aseptic loosening, a major clinical issue, characterizing by peri-implant fibrosis.** Our data suggest that the spindle-shaped, fibroblast-like cells in the fibrotic tissue of this POP aseptic loosening model, are mainly cells actively expressing LepR. **(Figure 2.a, Figure 2.c)**. Furthermore, these cells are ALPL+ ADIPOQ- **(Figure 3.b and c)**, suggesting that they are of the osteogenic subset of LepR+ cells. Further studies will be needed to elucidate the mechanism behind the differentiation of these mesenchymal stem cells into pro-fibrosis fibroblasts or pro-bone osteoblasts phenotype.

**SIGNIFICANCE: The identification of the osteogenic subset of LepR-repressing cells provides potential target candidate for the prevention and treatment of aseptic loosening of both POPs and TJRs.**

**REFERENCES:** 1, Fischer H. et al. Congr Res Serv., 2015. 2, Branemark R. et al. BJJ 2014. 3, Nicholson, J.A. et al., Injury, 2021. 4, Prock-Gibbs, H et al., JBJS, 2021.

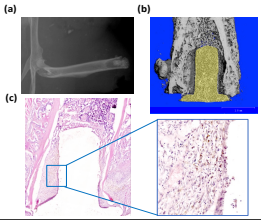


**Figure 1: Peri-implant radiolucency shown in radiograph (a) and microCT images in a percutaneous osseointegrated prosthesis (POP) aseptic loosening model. Histology images (H&E) showing peri-implant fibrotic tissue and the presence of elongated cells in it.**

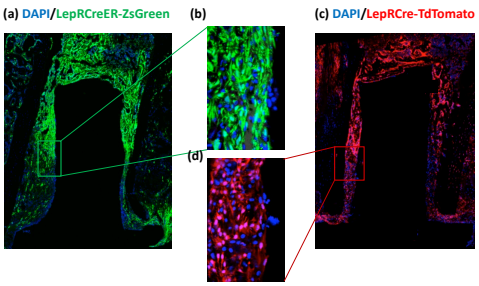


**Figure 2: LepR-expressing (a and b) and LepR-lineage cells (c and d) are abundant in the peri-implant fibrotic tissue shown with fluorescence images 2 weeks after surgery inducing aseptic loosening of POP.**

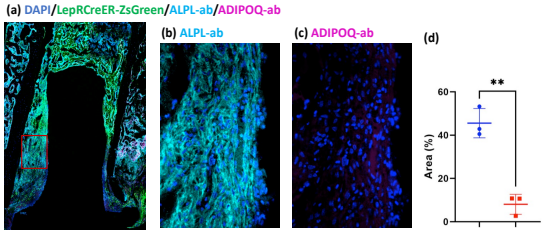


**Figure 3: LepR-expressing cells in peri-implant fibrotic tissue are ALPL+ and ADIPOQ- shown with fluorescence images (a, b, c). The percentage of LepR+ALPL+ area is significantly higher than LepR+ADIPOQ+ (d)**