INTRODUCTION: Angiogenesis and osteogenesis are essential for bone growth, fracture repair, and bone remodeling. VEGF has an important role in bone repair by promoting angiogenesis and osteogenesis. Our previous studies have demonstrated that fibroblast cell-based VEGF gene transfer\(^1\) and EPC therapy\(^2\) result in increased osteogenesis and angiogenesis in segmental bone defect models. In this study, the application of local EPC therapy to a segmental bone defect resulted in increased VEGF expression during the early period of fracture repair. Angiogenesis is a process that is critical for early callus formation and fracture healing. Increased VEGF expression resulting from local EPC therapy may be one of the mechanisms by which bone healing is enhanced with the use of EPCs. The results of this study support further investigation of EPC therapy for fracture healing.

METHODS: Rat bone marrow-derived EPCs were isolated from the rat bone marrow by the Ficoll-paque gradient centrifuge technique. The EPCs were cultured for 7 to 10 days in endothelial cell growth medium with supplements (EGM-2-MV-SingleQuotes, Clonetics), and collected for treatment of the rat segmental bone defect. EPCs were identified by immunocytochemistry staining with primary antibodies for CD34, CD133, Flk-1, and vWF. A total of fifty six rats were studied. A five millimeter segmental bone defect was created in the middle 1/3 of each femur followed by mini plate fixation. The treatment group received \(1 \times 10^6\) EPCs locally at the bone defect and control animals received saline only. Seven control and seven EPC treated rats were included in each group at 1, 2, 3 and 10 weeks. Animals were sacrificed at the end of the treatment period, and specimens from the fracture gap area were collected and immediately frozen. Rat VEGF mRNA was measured by reverse transcriptase-polymerase chain reaction (RT-PCR) and quantified by VisionWorksLS. All measurements were performed in triplicate.

RESULTS: Cultured EPCs at 1 week showed positive staining for CD34, CD133, Flk-1 and vWF markers (Figure 1). The EPC group had a greater VEGF expression than the control group at week 1, 2 and 3 but not at week 10. Three VEGF isoforms were detected in this rat model: VEGF\(^{120}\), VEGF\(^{164}\) and VEGF\(^{188}\). VEGF\(^{120}\) and VEGF\(^{164}\) levels peaked at two weeks, while VEGF\(^{188}\) levels peaked at three weeks. All three VEGF isoform levels were low at ten weeks (Figure 2).

DISCUSSION: EPC-based therapy for a segmental bone defect results in increased VEGF expression during the early period of fracture repair. In addition, the specific VEGF isoform may be a key regulator of the bone healing process. These findings demonstrate that EPCs promote fracture healing by increasing VEGF levels and thus stimulating angiogenesis, a process that is essential for early callus formation and bone regeneration.